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Siegfried Wartewig

IR and Raman Spectroscopy

IR and Raman Spectroscopy: Fundamental Processing. Siegfried Wartewig
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An Interactive Course

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IR and Raman Spectroscopy

Fundamental Processing

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Preface

At present, vibrational spectroscopy is undergoing a renaissance stimulated by many new developments in infrared and Raman instrumentation, such as high sensitive detectors, charge-coupled devices (CCD) and array detectors, laser excitation sources, step-scan technique, photoacoustic detection, spectral depth profiling, light-fiber optics, mapping and imaging in the field of microscopy, time resolved and surface-enhanced methods – in order to mention a few of these significant improvements. Of course this progress in vibrational spectroscopy is closely connected with the enormous development in computer technique. These developments have created novel applications of IR and Raman spectroscopy in various scientific disciplines ranging from chemistry and physics to bioscience and medicine.

On the other hand, handling, manipulating and evaluating of IR and Raman spectra, to say nothing of interpretation, remain as an old problem. For this purpose modern spectrometers are equipped with relevant powerful software packages. However, according to our experience it takes time to be familiar with all tools of such spectroscopic software. That is why the objective of this interactive course as interplay of text, software and spectra data is to teach you in fundamental manipulating and evaluating of vibrational spectra. It is the first volume in the WILEY-VCH series *Spectroscopic Techniques*, which deals with fundamental processing of IR and Raman spectroscopy. Certainly, further volumes covering other topics of practical vibrational spectroscopy will follow in future.

The audience for this interactive course should mainly include graduate students and technicians who are newcomers to IR and Raman spectroscopy. Hopefully, this book will also be of much benefit for practitioners in the daily work of a spectroscopic laboratory.

Leipzig, March 2003 *Siegfried Wartewig*

Acknowledgments

First and foremost, I thank my wife for her patience and understanding during the long process of writing this book.

I would like to express my gratitude to BRUKER OPTICS for their interest in this project and providing the software OPUS. I am deeply indebted to Dr. Heinrich Somberg for many helpful discussions.

Finally, I appreciate the efforts of Dr. Gudrun Walter, Wiley-VCH, with preparing this book. Rosemary Whitelock did a great job to improve the English.

1 Introduction

Nowadays, many analytical laboratories are equipped with an infrared (IR) and a Raman spectrometer, be it a dispersive device or a Fourier transform (FT) instrument. Raman and IR spectra provide images of molecular vibrations that complement each other and thus both spectroscopic techniques together are also called vibrational spectroscopy. The concerted evaluation of both spectra gives more information about the molecular structure than when they are evaluated separately.

Over the last years, there has been tremendous technical improvement in Raman and IR spectrometer design. For a newcomer to the field of vibrational spectroscopy, a modern spectrometer, whether it is a routine or a research-grade instrument, looks like a black box driven by a personal computer with very complex software. It is now state of the art that every spectrometer manufacturer provides specific software packages for data acquisition, both controlling the spectrometer and manipulating and evaluating the spectra. Often such software looks so sophisticated that this can be frustrating, not only for a newcomer. This book is intended to help you to overcome this problem and to understand the fundamental processing of vibrational spectra. It should enable and encourage you to process your data according to your own special needs.

The approach of the text is from the perspective of a spectroscopist involved in the daily work of a laboratory. The basis is the spectroscopic software OPUS developed by Bruker Optik GmbH (Ettlingen, Germany). The acronym OPUS stands for "OPtical User Software" on the Microsoft Windows platform. A demo version of OPUS including a collection of IR and Raman spectra is supplied on CD-ROM. In addition, you will find an IR library and a Raman library on your OPUS CD containing 350 and 250 entries, respectively. It is obvious that a demo version is not suitable for performing measurements. It is also not possible to export or import data files. Nevertheless, you have an "OPUS Workstation" at hand, which allows you to enter into the realm of data handling in vibrational spectroscopy. In order to make the work easier we keep to the approved rule "Learning by Doing". You will find a huge amount of illustrations and examples in the text, which you can and should verify by using your OPUS workstation. In this context, we will consider both IR spectra and Raman spectra in the same way.

The intent was not to write a new textbook on vibrational spectroscopy. So, we will consider neither band assignments and interpretation of spectra nor special

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experimental techniques. There are many excellent books available detailing the theory and experimental technique of IR- and Raman spectroscopy as well as their manifold applications in various fields. The reader interested in those topics is referred to the bibliography. However, in order to understand the functions of OPUS we will outline the basic principles of vibrational spectroscopy and explain the essential points of Fourier transformation.

Oriented on the menu of OPUS the book is divided into 13 chapters.

Chapter 2 deals with your personal "OPUS Workstation", its technical requirements, the software, and spectra database supplied on CD-ROM and how to install it.

Chapter 3 introduces the basic functions of OPUS. Here you will find general information about the OPUS windows and the handling of the data files. Once you have worked through this chapter you will be able to use the *Browser*, load and unload a spectrum into the *Spectrum Window*, select a spectral range, and deal with a *Report Window.*

Chapter 4 briefly summarizes the background of molecular vibrations, which is fundamental to an understanding of vibrational spectra. Here we will also explain the difference between IR and Raman spectroscopy.

Chapter 5 addresses the concept of the Fourier transform technique without going into mathematical details. The advantages of the technique and the artefacts connected with it are discussed. These include such issues as apodization function, zerofilling, phase correction, and acquisition mode, which are important for an understanding of the measuring process. For Raman spectroscopy, the 'rivalry' between dispersive and FT techniques is also considered.

Chapters 6 to 9 cover the details of data managing: handling, editing, and displaying of OPUS files in the form of spectra and reports.

Chapters 10 and 11 form the most exciting and important part of this book and deal with all aspects of manipulation and evaluation of spectra.

Chapter 12 covers the options for manipulating the display of spectra.

Finally, *Chapter 13* outlines how to print spectra and reports.

The book profits from a huge number of figures together with the great advantage that you can create and verify most of them on your own PC screen. It is the combination of written text, the software tools, and the spectra supplied, which make it different from other books on vibrational spectroscopy. It is strongly recommended that you use all these educational tools in a complementary and interactive way, switching from the textbook to the software tools and the set of spectroscopic data stored on your PC and back again. Following this interactive line you will soon improve your skill in processing vibrational spectroscopic data.

2 Your Personal “OPUS Workstation”

2.1 Technical Requirements

In order to install and run the OPUS version 4.0 demo you will need a Pentium III class PC with the Microsoft WINDOWS NT 4.0, WINDOWS 2000 or WINDOWS XP platforms, but note that neither WINDOWS 95 nor WINDOWS 98 are suitable. It is recommended to use an 800 MHz (or higher) processor with at least 128 MB RAM base memory (256 MB recommended) and a hard disk. The demo version of the software and the spectra database are both stored on the CD-ROM enclosed. A corresponding CD-ROM drive is therefore required. Prior to starting the installation procedure you should ensure that the operating system WINDOWS NT 4.0 with the Service Pack 6 or WINDOWS 2000 or WINDOWS XP is already installed on your computer. The minimum technical requirements for your OPUS workstation are summarized in Tab. 2.1.

Table 2.1. Minimum requirements for your OPUS workstation.

Description	Field Information
Operating System	Microsoft Windows NT version 4.0, Microsoft Internet Explorer 5, Service Pack 6 or Microsoft Windows 2000 Professional, standard configuration or Microsoft Windows XP Professional
CPU	Intel Pentium III 800 MHz or higher
Hard Disk	800 Mbytes free space or more
Memory	128 Mbytes or better
Monitor	15 in SVGA or better
Network/LAN	Ethernet (10 or 10/100 MHz)
Interfaces	Parallel (1), RS232 (1 for serial mouse)
Floppy drive	3.5 in
CD ROM	4X
Mouse	PS/2 or serial
Keyboard	
Graphics card	Resolution 600 x 800 or higher, 8 Mbytes RAM

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2.2 Installing OPUS

In order to start the installation of OPUS insert the OPUS CD into your CD-ROM drive; the OPUS installation program will start automatically. Depending on your hardware this may take several seconds. Do not attempt to start the installation manually during this period or the installation may fail. The installation program will guide you step by step through the necessary procedure. You only need to follow the instructions shown in the different dialogs. In the first dialog box appearing you can choose the language you wish to use (see Fig. 2.1). Confirm your choice by clicking on the *OK* button.

In the following dialog box shown in Fig. 2.2 the installation set-up will ask you for the path in which the OPUS program should be stored. The default destination folder is C:\OPUSDEMO. Of course, you may choose another folder using the *Browser* button to browse your hard disk. After clicking on the *Next* button, the installation program will begin to copy the files from the CD to your hard disk. The progress of this installation process is displayed by four status bars as illustrated by the snapshot in Fig. 2.3. Finally, the *Set-up Complete* box shown in Fig. 2.4 appears, in which you should check "*Yes, I want to restart my computer now"* and then click on the *Finish* button. After re-booting and successful installation, the program icon of OPUS will be added to the Windows Start menu as depicted in Fig. 2.5. Of course, you can also create the OPUS icon on the desktop as usual in the Windows environment.

In the directory C:\OPUSDEMO\DATA you will find the collection of vibrational spectra split up into the folders ACQUIS, APODIZ, MIR, NIR, and RAMAN. The two spectra libraries IR and Raman are stored in the directory C:\OPUSDEMO\Library.

2.3 Starting OPUS

You can run the OPUSDEMO program most conveniently by clicking the OPUS icon from the "Start" menu. After starting the program, the login dialog box shown in Fig. 2.6 appears. Notice that running OPUSDEMO does not require a password

In the login dialog box, click on *Assigned Workspace* to view a list of available user profiles. A workspace file is marked with the extension ".ows". The demo version provides four different workspaces. The workspaces NIR.ows and RAMAN.ows are particularly devoted to NIR spectroscopy and Raman spectroscopy, respectively. All functions of OPUS are available using default.ows. The profile DEMO.ows is especially created for the issues of fundamental processing, which we will discuss in this book. Generally, OPUS as well as OPUSDEMO allows you to generate your own workspace that best meets your wishes. However, in OPUSDEMO you cannot save this new profile. Therefore, we recommend selecting DEMO.ows.

After confirming the login dialog the OPUS registration screen will be displayed, showing the version number and the available software packages

Figure 2.1. The Set-up program: The language dialog box.

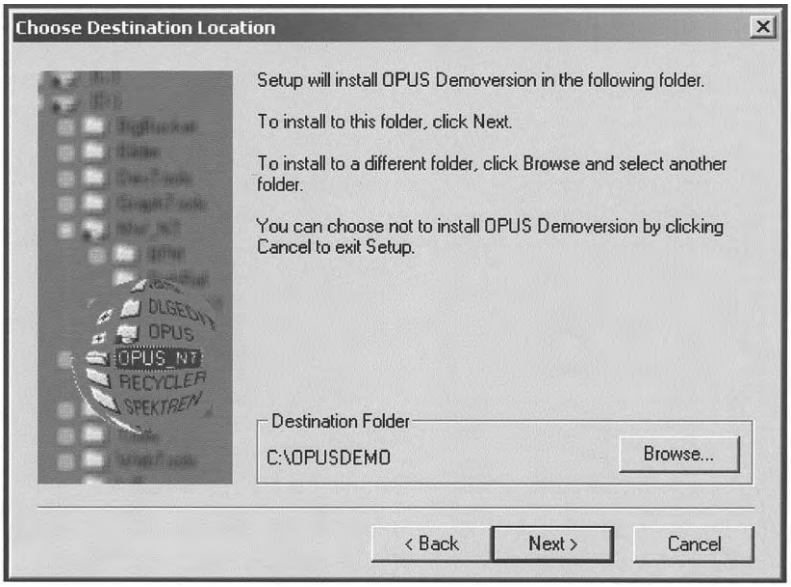

Figure 2.2. The Set-up program: Set installation path for OPUS.

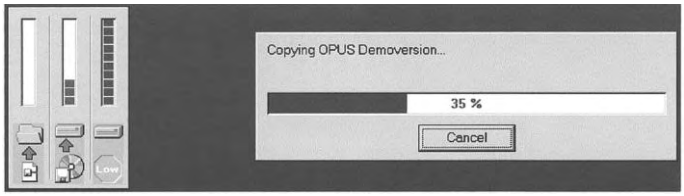

Figure 2.3. Status bars indicating the progress of installation.

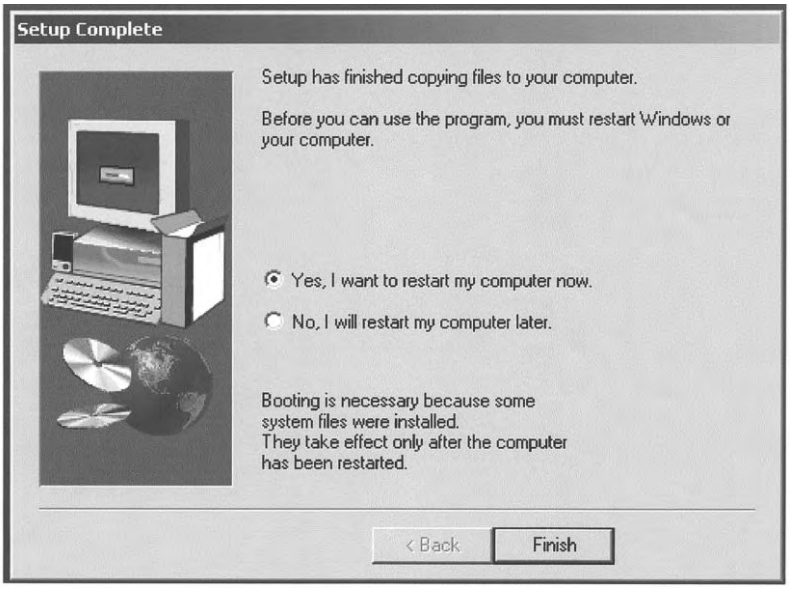

Figure 2.4. The *Set-up Complete* dialog box.

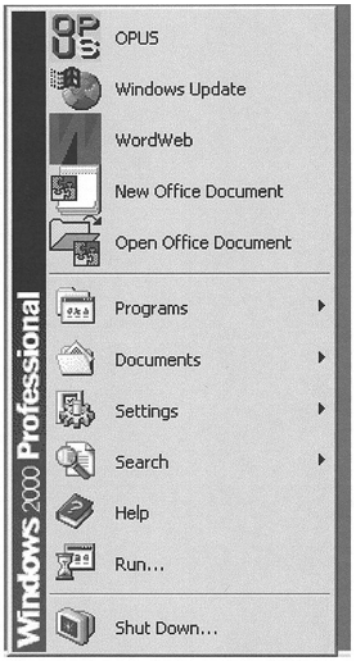

Figure 2.5. The Windows Start menu with the OPUS icon added.

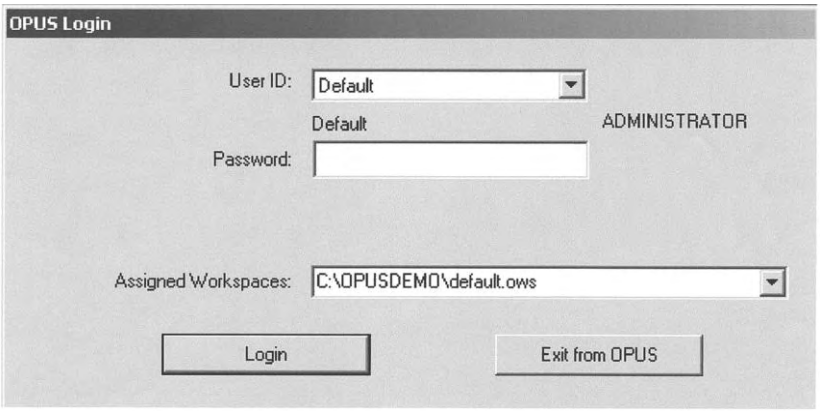

Figure 2.6. The OPUS login dialog box.

(see Fig 2.7). In addition to the basic program, OPUSDEMO provides specific packages as follows.

OPUS 3D contains functions to display and manipulate 3D files. These files can be the results of time resolved measurements, other hyphenated techniques or mapping experiments.

OPUS IDENT is a software package designed to identify substances by their vibrational spectra and is mainly used for quality control.

OPUS QUANT is a package for the quantitative evaluation of spectra.

OPUS SEARCH is a package for the identification of unknown substances using a library search for the spectrum, chemical structure information or individual bands.

OPUS SEMI is a special package for the spectroscopic analysis of semiconductors, including oxygen and carbon analysis in silicon.

VALIDATION is software that helps users comply with the regulation *"21 CFR Part 11 Electronic Records; Electronic Signature"* issued by the United States Food and Drug Administration, "FDA" (1997).

The topics of these additional packages are beyond the scope of our interactive course. However, you can try to apply them using the introductions to these items that you can find via the menu *Help*.

After clicking once again on the *OK* button the standard OPUS user interface will be displayed as depicted in Fig 2.8. It consists of three windows, the browser window, the spectrum window, and the overview. If you place the cursor on the border between these windows, you will notice the cursor changes to ⇹ or ⇳. This allows you to re-size the windows.

On the top of the user interface, there is a line of the menus and under them the icon bars. By left-clicking on a menu button you have access to a corresponding context menu, also known as pull-down menu. The icon bars and the menu items will be explained in detail in the following chapters.

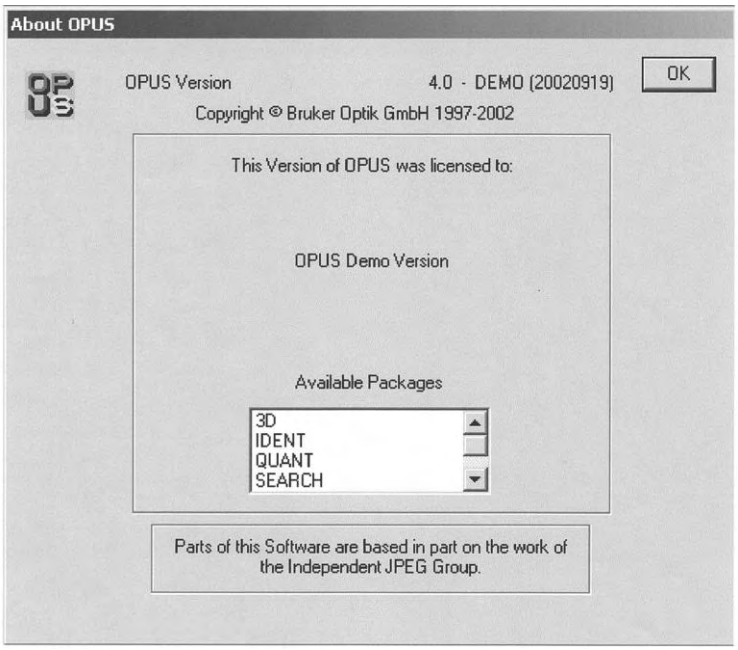

Figure 2.7. The OPUS information screen.

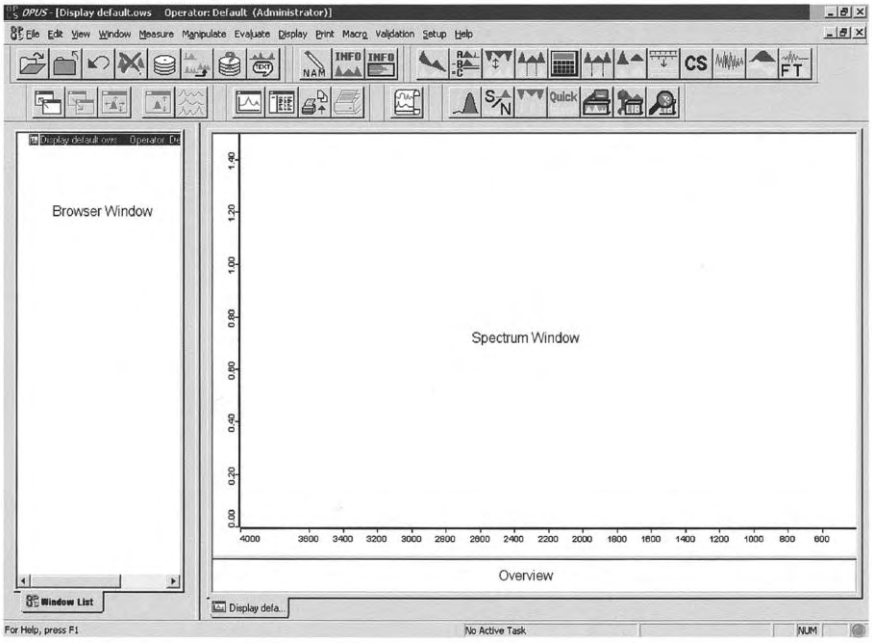

Figure 2.8. The OPUS user interface.

3 OPUS Basics

This chapter introduces the basic functions of OPUS. Here you will find general information about the OPUS windows and the handling of data files. Many shortcuts and dialog boxes of OPUS are identical with or resemble those of Windows. If you have already gained experience with Windows, you will find it easy to use OPUS. So OPUS applies drag and drop as well as keystroke shortcuts, like copy *(CTLR* C), cut *(CTRL* X), and paste *(CTRL* V).

To use these shortcuts, you first have to select a part of the text or select the file you want to process and then apply the shortcut. Drag and drop simplifies the copy and paste process, but works only with files. You have to left click on the file icon in the Browser and keep the left mouse button pressed. Then move the file to the dialog box or the window, which performs the desired function. Upon releasing the mouse button, the file will automatically be loaded into the dialog box.

Dialog boxes often consist of several pages, between which you can switch by left clicking on the tabs of the page. An example of a dialog box is given in Fig. 3.1.

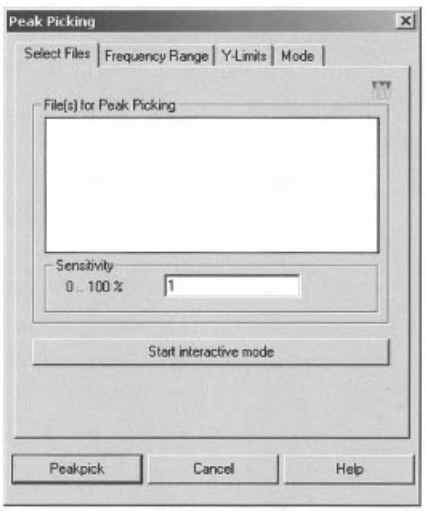

Figure 3.1. An example of a four-pages dialog box.

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Buttons and menu items are only active if they are displayed in black; gray buttons are not active.

3.1 Loading and Selecting a File

You can load a file into the OPUS user interface (see Fig. 2.8) by using the *Load File* command from the *File* menu or by clicking on the icon located in the upper left icon bar. In this manner you open the *Load Spectrum* dialog box shown in Fig. 3.2. The icons in the upper line of this box have the following meaning:

- By clicking on you can choose a path to search for spectra.
- Goes to the last folder visited.
- Changes to the parent directory.
- Creates a new folder.
- By clicking on you open the *View Menu* for the files listed in the left window. You can select between *Large Icons, Small Icons, List, Details, and Thumbnails.* Test it to become familiar with this menu. We would recommend to use *List* or *Small Icons.*

Using the box *File name* you can also manually type in the name of the file you wish to load.

In the last three boxes additional information contained in OPUS files can be selected by choosing parameters. The contents or values of these parameters will be displayed on the right hand side of the parameter. Keep in mind that not all OPUS files contain additional information. Figure out by yourself which parameters are accessible in this way.

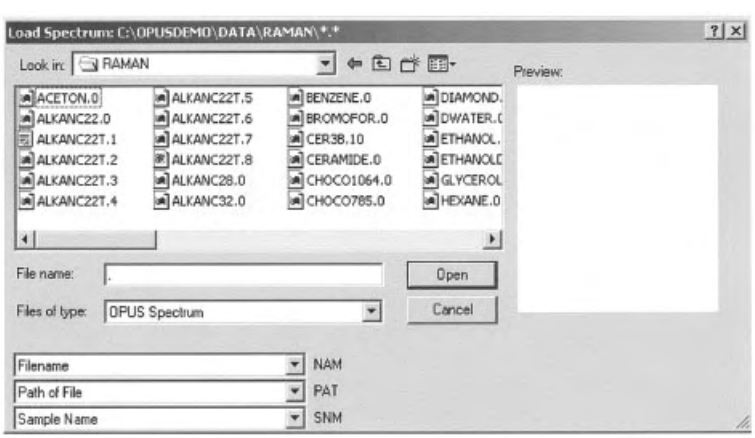

Figure 3.2. The load file dialog box: No file selected.

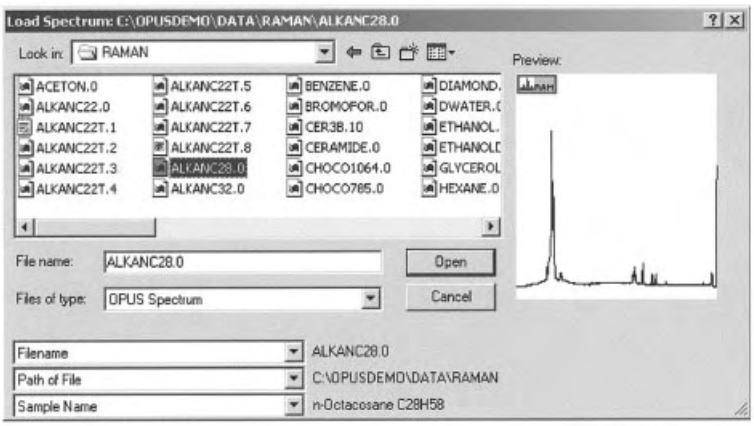

Figure 3.3. The load file dialog box with active preview.

In Fig. 3.2 no file has yet been selected, therefore the abbreviation of the respective parameter will be displayed.

By selecting a file, here the file RAMAN\ALKANC28.0, the *Load Box* will change as shown in Fig. 3.3. The right window shows a brief preview of the selected spectrum without the axes, and the data blocks belonging to the file are shown in the top left corner as small icons. The spectrum parameters now appear below the spectrum.

You can select several items in the file list using the control or shift key while selecting the spectra. In this case the number of selected files will be shown instead of the data blocks and spectra previews. Clicking on the *Open* button will load the spectrum into OPUS user interface and automatically close the *Load* Box.

Another way of loading a file is by "dragging" it from the Windows Explorer into the display window or the Browser window. Using this version of loading the background of the display icon in the Browser window becomes green.

An OPUS file can consist of various data blocks; several of them are listed below:

- Absorbance spectrum
- Transmittance spectrum
- Single channel spectrum of the reference
- Single channel spectrum of the sample
- Raman spectrum
- Interferogram of the sample
- Audit Trail
- Information

- INTEG Integration report
- FIT.REP Fit report
- SEARCH Search report
- PEAKS Peak table
- REPORT Report

We will consider the data blocks in detail in the following chapters.

You will encounter the *File Selection Page* in various dialog boxes; its purpose is to choose a file on which an OPUS function or command will be performed. As an example, Fig. 3.4 shows the file selection page of the *Baseline Correction c*ommand.

The file(s) you want to process are to be entered into the *File(s) to Correct* list. You have several possibilities for loading a file into the selection box:

- Pre-select one or more files before opening the relevant selection box command; these files will automatically appear in the selection box.
- Double-click on a spectrum block while the selection box is open.
- Use the Browser to drag and drop a spectrum block of a file into the selection box. By pressing the control key you can select multiple files.
- Instead of selecting a spectrum block you can also drag and drop a file name into the selection box. This will select all data blocks contained in this file. Again use the control key to select multiple files.
- Drag and drop spectra directly from their spectrum window to the selection box.

When a data block cannot be processed by a function, it is not possible to load this file into the selection box. For instance, the conversion AB to TR is not applicable to an interferogram. Sometimes, the parameters of an OPUS function (on the following page of the functions dialog box) have to be set prior to load-

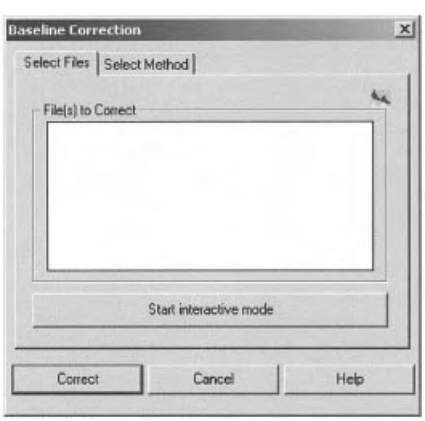

Figure 3.4. An example of the files selection page.

Figure 3.5. The convert spectra dialog box indicating parameter mismatch.

ing a file, in order to be able to select the file. When the selected data block can in principle be processed by the OPUS functions, but the parameters do not match the data, the file name in the selection box will be indicated in red and the warning symbol ⚠ will be displayed (see Fig. 3.5).

To remove files from the selection box first highlight them. Select several files by holding down the *Shift* or *Control* key while you click on the files with the left mouse button. Then use the *Delete* key to remove the files chosen.

Each and every operation dealing with a file, be it a manipulation or evaluation, will automatically be documented and added as audit trail to the file in terms of the data block HISTORY. You have access to this "history" report by right-clicking on this icon.

3.2 The Browser Window

The design and functionality of the Browser resemble those of Windows Explorer. If you load an OPUS file, the file name, the data blocks, and the file status information become visible in the Browser window (see Fig. 3.6a).

It is possible to display several spectrum windows (see Fig.3.6 b). If you have opened several spectrum windows you can toggle between them by left-clicking on the display icon. Furthermore, clicking on ⊟ will collapse the respective spectrum block, as shown in Fig. 3.6c, and clicking on ⊞ will restore it.

In the Browser window, a blue file icon of the file precedes the file name. The blue color indicates that the file has not yet been processed. Next to this box are the file name and a number, indicating the copy of the loaded file. Notice that you can load a file several times into the Browser.

The data block bar represents all types of information contained in the OPUS file. In Fig. 3.6a the icons RAM and Sig indicate a Raman spectrum and an inter-

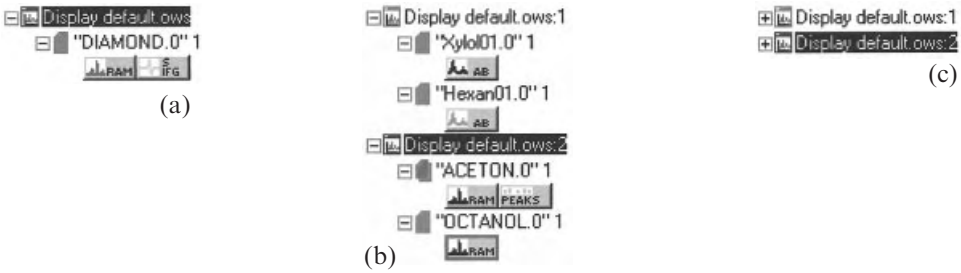

Figure 3.6. The Browser file list: (a) one file loaded; (b) several files loaded; (c) file blocks collapsed.

ferogram, respectively The first data block will automatically be displayed in the spectrum window. The data blocks can be selected in the same manner as you select files. Selected data blocks are shown framed in red. By left-clicking on the data block you can change the color of the displayed spectrum in the spectrum window.

If you have loaded several spectra, you can select the spectrum you want to process by clicking on the file name. If you hold down the *Shift* key while selecting two files, all files in between these two files will also be selected. If you use the *Control* key instead of the *Shift* key, all files you selectively click on will be selected. The same is true for data blocks.

If you load the same spectrum several times, the numbers following the file name will label the copies. The file name of the active spectrum will appear in a red box. The display icon can be used to toggle the spectrum windows. You can select all spectra in a spectrum frame by holding the *Shift* key while clicking on a file name. If the cursor is positioned on a file name, the user name, sample name, and the sample form will be displayed in a small extra window.

Using the right mouse button on a file name opens the pop-up menu shown in Fig. 3.7, which allows you to manipulate the file. You can save the changes you have made so far, remove the file from the browser, rename it or restore the original data with the undo function.

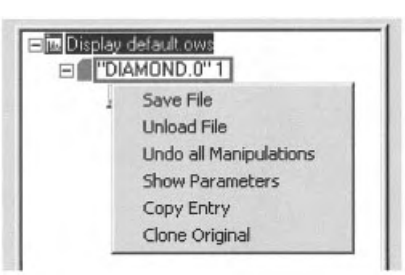

Figure 3.7. The pop-up menu for the file manipulation.

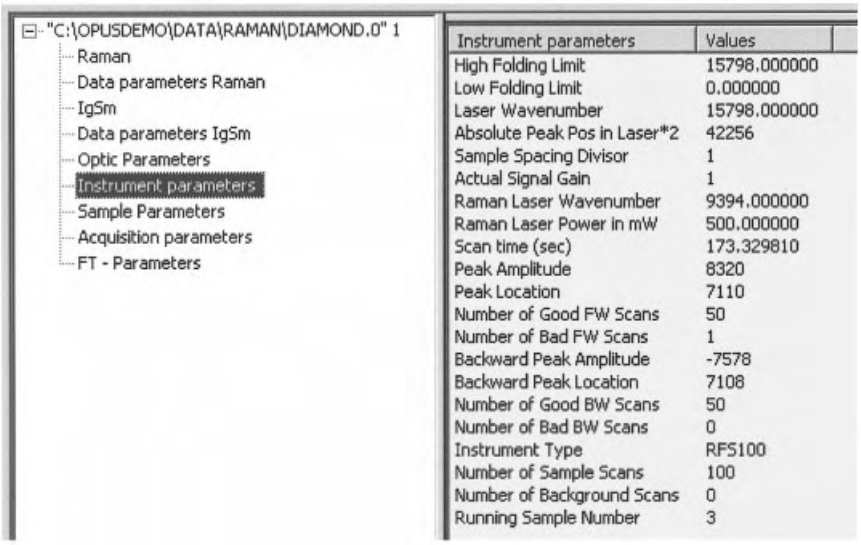

Figure 3.8. The list of instrument parameters.

The *Show Parameters* command displays all information about the data acquisition stored together with the file, namely the parameters of the sample, instrument, optic, acquisition, Fourier transformation etc. This is done in the form of a report window (see Chapter 3.4). An example of these parameters is illustrated in Fig. 3.8 for the Raman file DIAMOND. Load other files and look at the relevant data.

The last two commands, *Copy Entry* and *Clone Original*, are used to duplicate spectrum files. Use *Copy Entry* to make a copy of a data file that has been manipulated. *Clone Original* creates a copy of the original data of such a file.

If you right-click on a data block you have access to another pop-up menu, shown in Fig. 3.9. Herewith you can change the display color of the spectrum or remove it from the spectrum window. The other commands will be discussed later.

3.3 The Spectrum Window

When the default settings are loaded, the spectrum window is located in the right pane of the OPUS user interface. After a file has been loaded, all spectral features of this file such as spectra and interferograms can be displayed. As an example, the transmittance spectrum, the single channel sample spectrum, the sample interferogram, and the single channel reference spectrum of the MIR file INDIGO are displayed in Fig. 3.10.

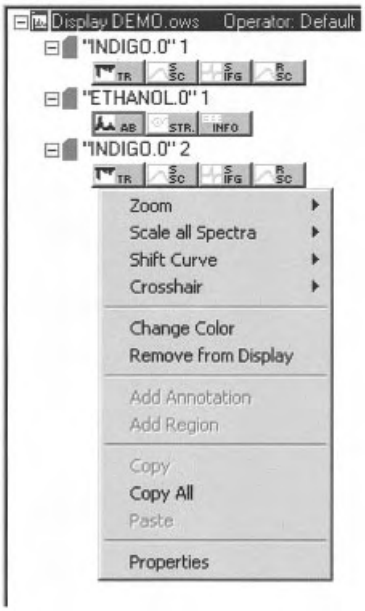

Figure 3.9. The pop-up menu for the spectrum manipulation.

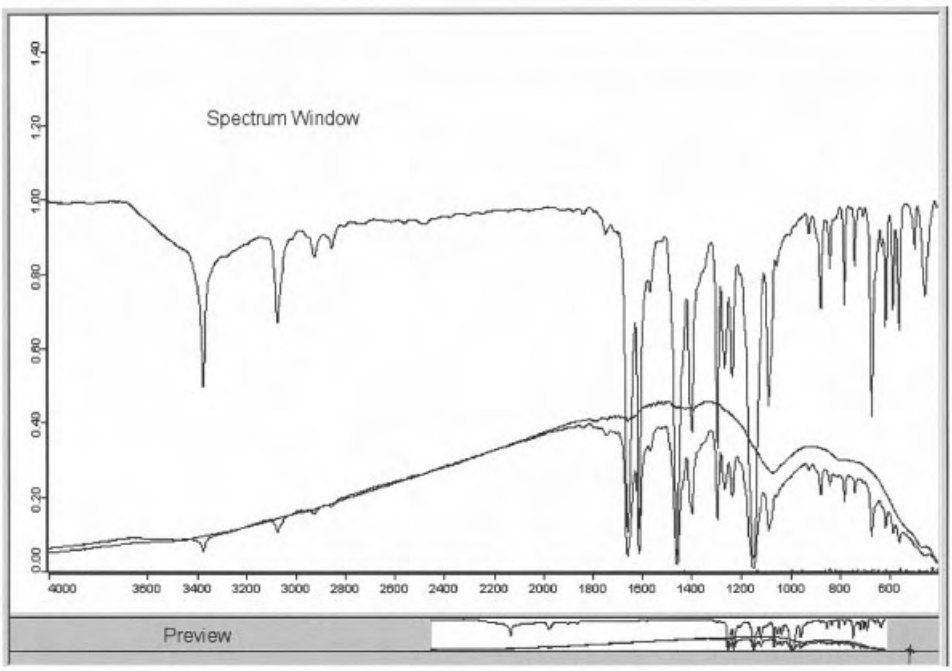

Figure 3.10. The spectrum window and preview window.

The default settings are the spectral range from 4000 to 400 cm^{-1} and 0 to 1.5 extinction units. However, the spectrum can be scaled to show the complete data range by using the *Display* → *Scale all* command or by clicking on the icon . In Fig. 3.10 the *Scale all* command was not applied. Therefore only the preview window shows the total spectral range of the data. In the overview, the part shown in the spectrum window is depicted on a white background, while the rest of the data range is grayed out. If you place the cursor on the white region, you will notice the cursor will change to ✥ . Now you can move the area of the displayed (white) region while pushing the left mouse button. On the other hand, if you position the cursor on the boundary of the white region, you will see the cursor will change to ↔ or ↕. Pushing now the left mouse button and moving it, you can change the size of the white region.

For illustration, in the frame of the default settings for Fig. 3.10 it is a little difficult to figure out the interferogram. But, if you use ↕ or ↔ in the overview window, you can change the display areas to best meet your needs.

If you position the cursor on the border between the preview and the spectrum window or on the border between the browser and the spectrum window, the cursor will change to ⇕ and ⇔, respectively. This allows you to re-size the windows.

Furthermore, the properties of the displayed spectra can be controlled in two other ways either by right-clicking on any point of the spectrum window or by right-clicking on any point of the spectral curve. In this manner, you will open the pop-up menus shown in Fig. 3.11(a) and (b), respectively, that allow you to access the property sheet of the spectrum as follows.

- The *Zoom* option allows you to choose the magnification. After selecting the *Zoom in* command, use the left mouse button to draw a frame around the region you would like to expand. After a second mouse click, the cross haired cursor disappears and then you can reposition the frame. Now left-click again and the area marked by the frame will be resized to the spectrum window. Apply the *Zoom out* command to decrease the magnification or switch back to the original view by selecting *Show Everything (XY)* from the *Scale all Spectra* command.
- *Shift Curve* allows you to move *(Whole Curv*e) or stretch *(Top*, *Botto*m) the spectrum along the *y*-axis. Note the command *Whole Curve* is only accessible after right-clicking on any point of the spectral curve and then placing the cursor in *Shift Curve* (see Fig. 3.11c). If *Whole Curve* is selected, left-click on any point of the spectral curve and holding down the mouse button you can move the curve as you want. When several spectra are displayed, you can repeat this procedure for each of the curves. Turn the *Shift* modus off with a click on the right mouse button. The *Reset* command revokes all changes.
- An extended menu is available for the *Crosshair* command. Selecting *Cursor* changes the shape of the cursor to crosshairs and the *x,y*-position is displayed in the top right corner of the spectrum window. The crosshair can be locked

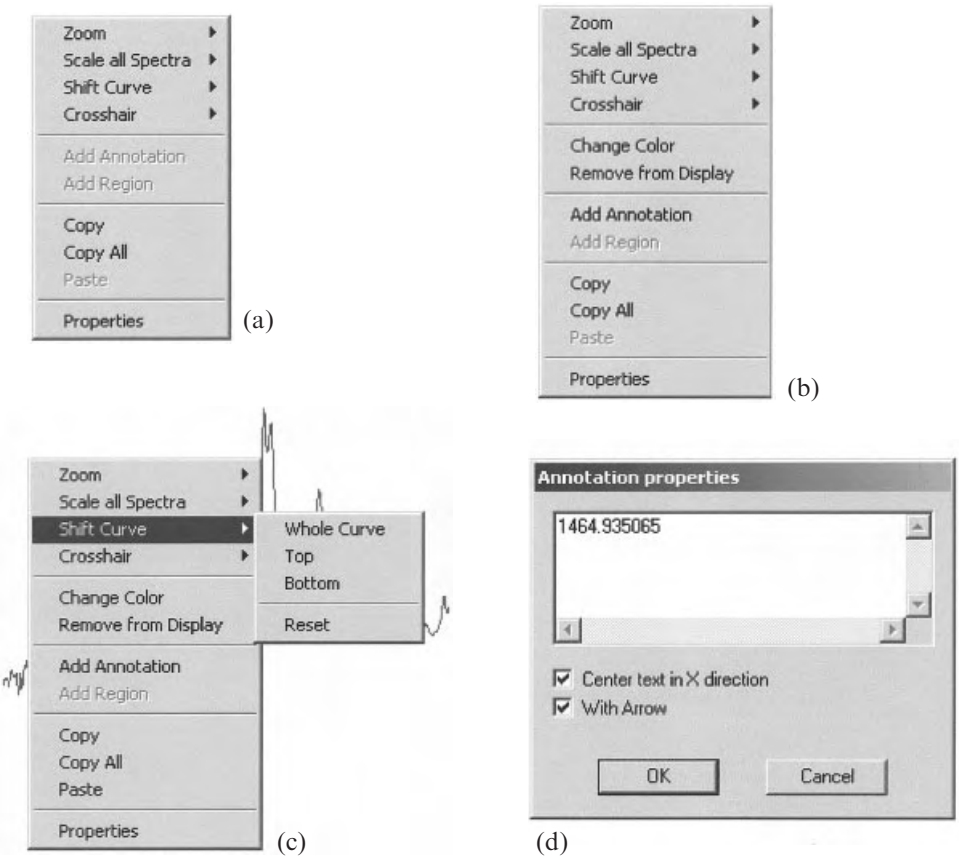

Figure 3.11. The pop-up menu to access spectrum properties: (a) opened by right-click on any point of the spectrum window; (b) opened by right-click on any point of the spectral curve; (c) opened by right-click on any point of the spectral curve and then the cursor placed in *Shift Curve;* (d) the *Annotation Properties* dialog box.

to follow the data points of a spectrum with the *Follow Data* command, allowing a comfortable read-out of the data points. Switch back to the regular cursor by a right-click.

- *Change Color* and *Remove from Display* are identical with the commands described in Section 3.2.
- You can label spectral features using the *Add Annotation* command. After activating the command, a click on a point of the spectrum will insert an arrow at the position of the cursor. The wavenumber of this data point will be inserted by default. Right-click on the label opens a further pop-up menu. If you click *Properties,* the dialog box *Annotation properties* appears (see Fig. 3.11d) where you can edit the label, e.g. add a text instead of the wave-

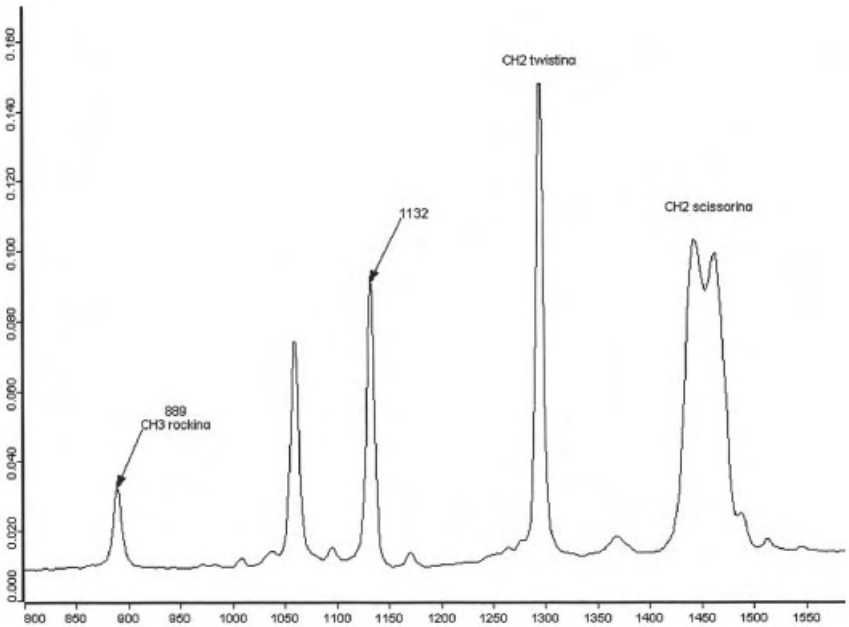

Figure 3.12. The Raman spectrum of n-docosane (file RAMAN\ALKANC22) with annotations.

number. Fig. 3.12 illustrates the various forms of annotation for the Raman spectrum of n-docosane. Performing this procedure yields the annotation data block attached to the file.

- *Copy* and *Copy All* enable you to copy one or all of the spectra into a different spectrum window via the clipboard. After performing these actions you have to open a new window or activate an existing one and then call up the pop-up menu of this frame and choose *Past*e.
- *Properties* opens the spectrum window properties dialog box shown in Fig. 3.13, 3.14, and 3.15.

On the *Display Limits* page you will find the controls for setting the limits of the displayed region. You can change the size to meet your preference.

Each spectrum can be depicted with additional axes on top and on the right side. This option you will find on the *Axes* page. If the spectrum window holds more than one spectrum you can have separate axes drawn for each spectrum if you choose X between. An example with two spectra is given in Fig. 3.16.

The default height and background color for the spectrum and overview window can be set on the *General* page. You can suppress to include spectra in a spectrum window which are recorded in units incompatible with the data de-

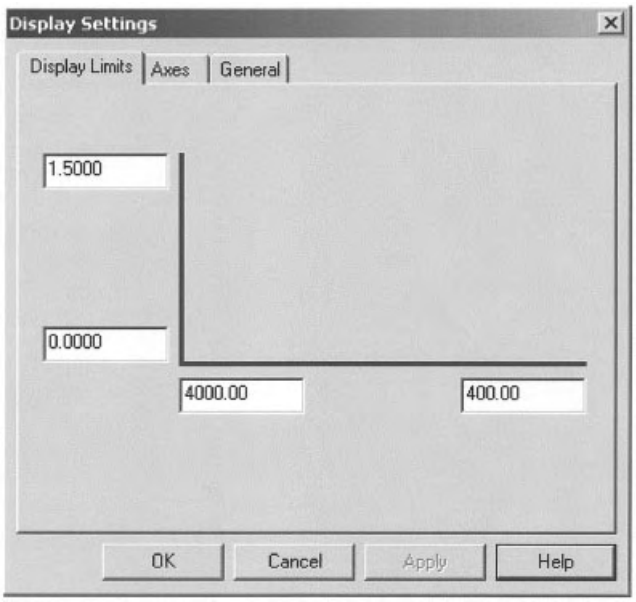

Figure 3.13. The *Display Settings* dialog box: *Display Limits* page.

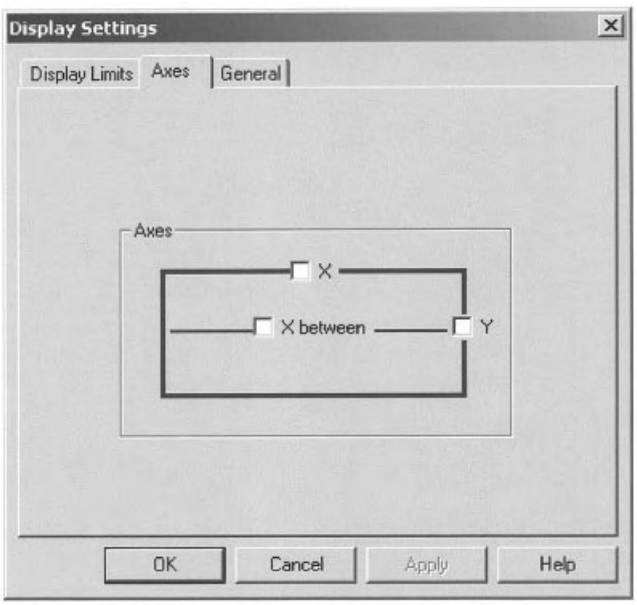

Figure 3.14. The *Display Settings* dialog box: *Axes* page.

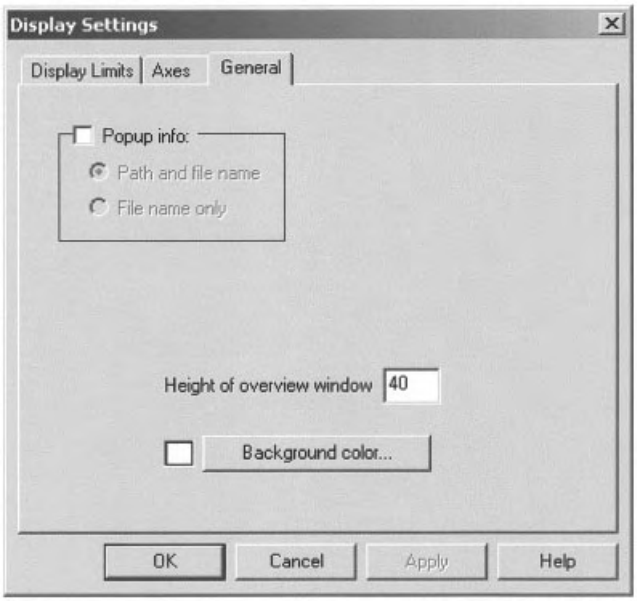

Figure 3.15. The *Display Settings* dialog box: *General* page.

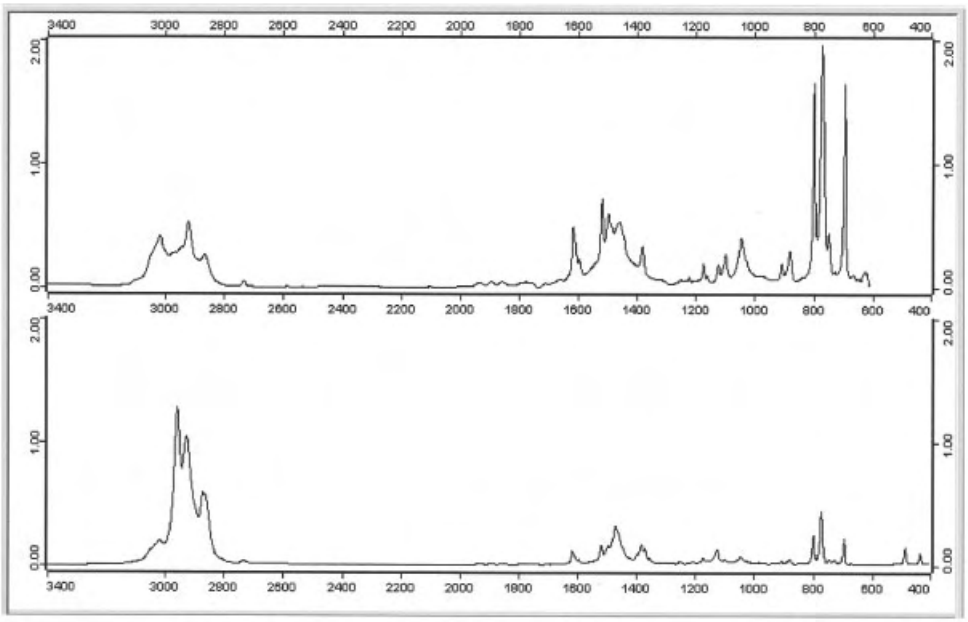

Figure 3.16. The presentation of two spectra.

picted first, e.g. data points and wavenumbers. You have the option of restricting the abscissa, the ordinate or both. In order to include spectra with incompatible units these boxes should not be checked.

3.4 The Report Window

Reports are used to display numerical or text information, typically the result of an evaluation or the parameters associated with a spectrum. Information of this type is stored in report blocks; double-clicking on a report block (exception: peak tables) will automatically open a report window. A blank report window can be opened from the *Window* menu (see Chapter 9). As an example, the report of the peak picking data for the Raman spectrum of n-docosane is shown in Fig. 3.17.

The information contained in the report block is displayed in the form of a tree on the left side of the report window. Highlight an item of the tree to display its data; if you click on the column title you can sort the data according to this column. If the block also contains a header, this information will be displayed in an extra window. The report window also has a *Properties* dialog box associated; see Fig. 3.18. Open this box by right-clicking on the displayed data of the report window. Furthermore, you can set the font and font size used for a printout of the report in the *Font* dialog box, Fig. 3.19, accessible by clicking on *Printer Font.*

3.5 Choosing a Spectral Range

Some OPUS functions require a spectral interval for data processing. Do not confuse: OPUS uses *wavenumber* and *frequency* synonymously. To be correct we prefer the term *wavenumber*. You can set the data range that will be processed by a given function in three different ways, see Fig. 3.20.

- Select the spectral range interactively.
- Use the range represented by the current spectrum window.
- Define the range numerically.

If you want to select the range interactively, load the spectra you want to process in the file selection page then go to the *Frequency Range* page and click on *Interactiv*e. A new window (Fig 3.21), similar to a spectrum window, in which the loaded spectrum is displayed will open. Depending on the x start- and end-points, the spectrum will exactly fit the window or a wider spectral range will be displayed. In the latter case, the part that contains no data is depicted in gray. Should you only see a gray window with no data then you will have to close the window again and choose different values for the start- and end-points.

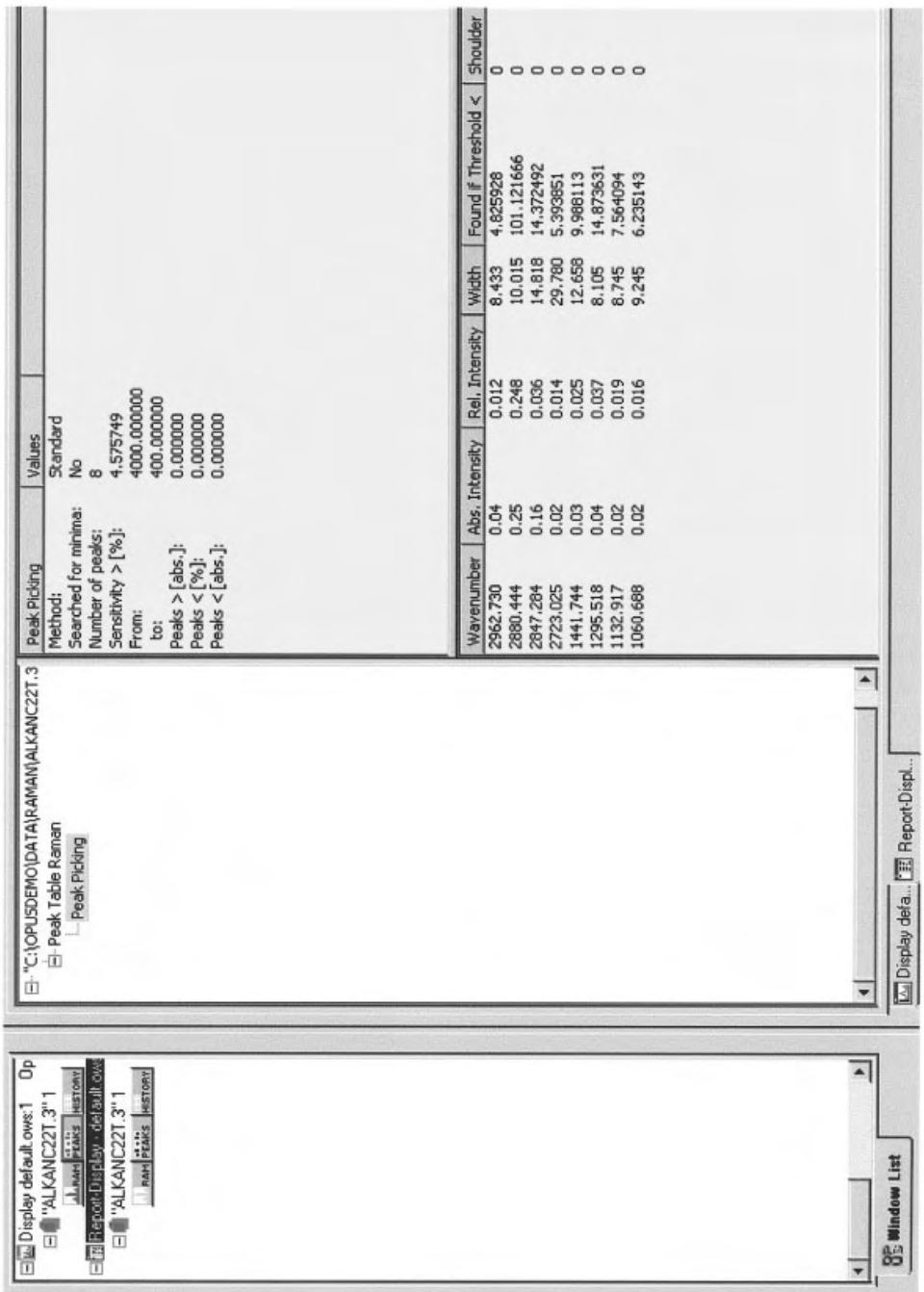

Figure 3.17. An example of a report window: *Peak Picking*.

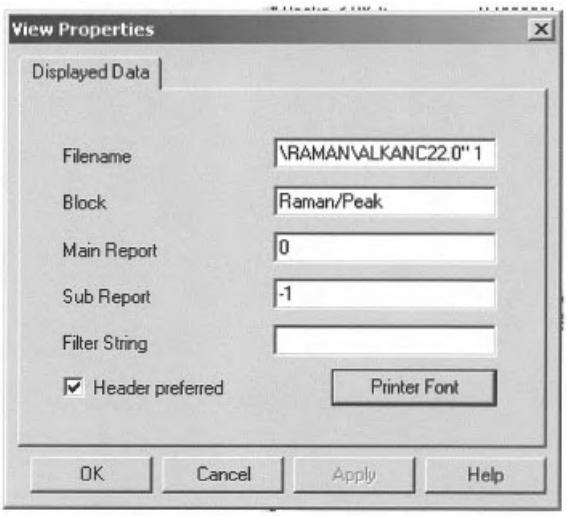

Figure 3.18. The *View Properties* box of the report window.

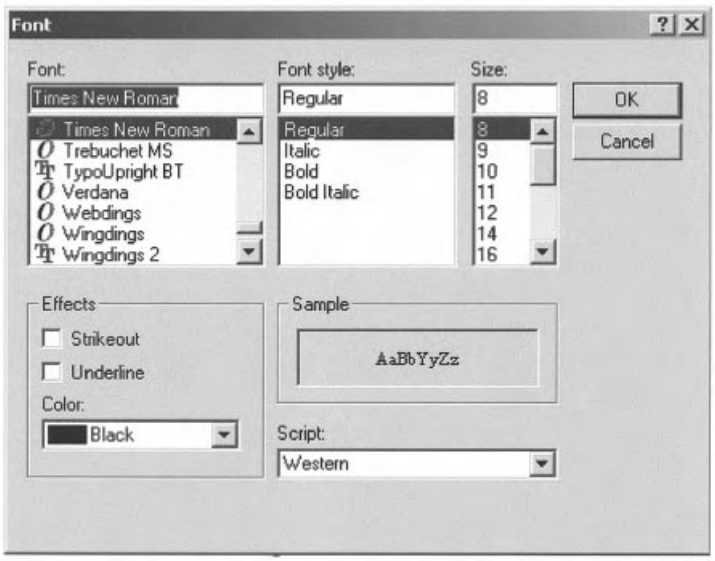

Figure 3.19. The *Font* dialog box.

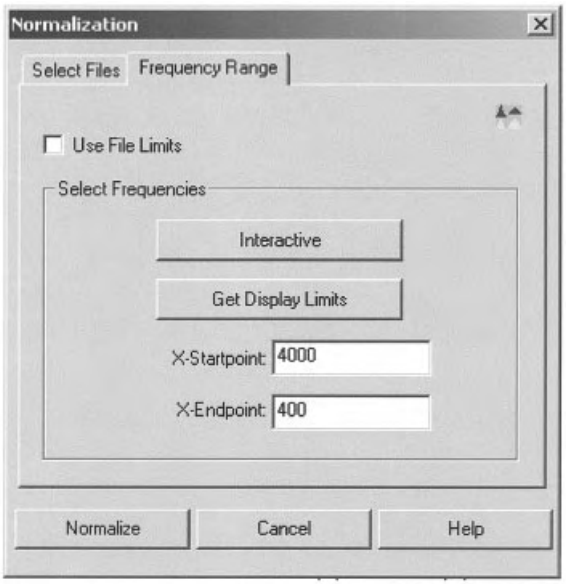

Figure 3.20. A *Wavenumber Range* selection box.

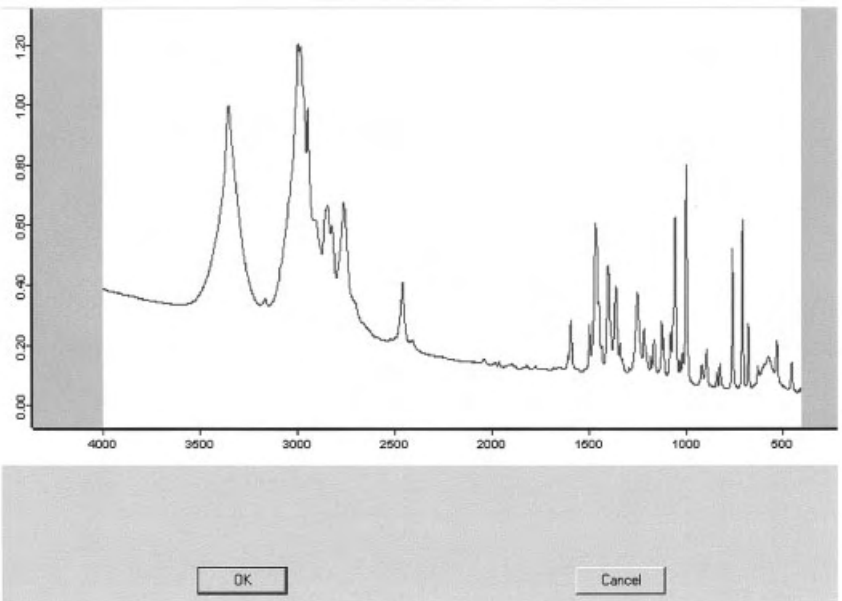

Figure 3.21. Choosing wavenumber range interactively.

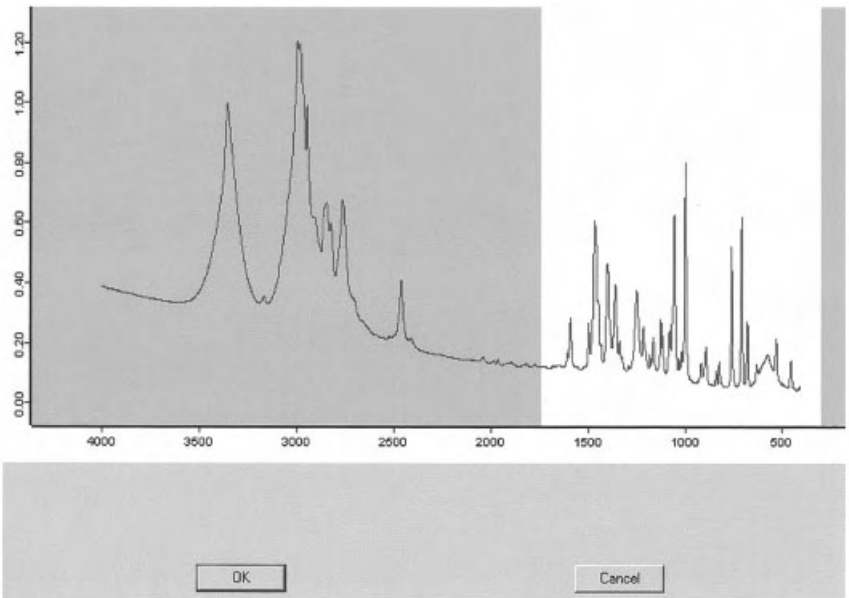

Figure 3.22. Choosing wavenumber range interactively: The spectral interval is narrowed.

If you left-click on the left or right window border (the cursor changes to ↔) and drag the mouse, you can narrow the wavenumber range, as a part of the spectrum will be grayed out (see Fig. 3.22). Only the remaining white region will be used for data processing. After you have narrowed the spectral interval down to the region of interest, you can also move the white window in the same manner by clicking and moving the mouse. As you do so the cursor will change to ✥.

By clicking *OK* you confirm the use of the selected interval for data processing.

3.6 The Toolbars

OPUS toolbars offer shortcuts to the most commonly used functions. If the cursor rests for a couple of seconds on a shortcut icon, a tool tip summarizing the icons function will be displayed. In addition, the icons can be found listed next to their function on the pop-up menu.

The toolbar configurations are stored with the workspace settings in a file with the extension .ows. Details of how to configure the toolbars can be found in Section 8.1. As mentioned above, for the purpose of our interactive course we recommend that you choose the workspace DEMO.ows.

4 Basic Principles of Vibrational Spectroscopy

4.1 Molecular Vibrations

The easiest way of modelling molecular vibrations is to imagine the atoms in a molecule as balls, and the chemical bonds connecting them as massless springs. Such a ball-and-spring model for a diatomic molecule is illustrated in Fig. 4.1. Let us assume that the masses of the two atoms are m_1 and m_2, respectively, and that the restoring force F of the spring is proportional to the displacement x of the atoms from their equilibrium position

$$F = -kx \tag{4.1}$$

where k is the force constant of the spring, in N m^{-1}, which is a measure of the strength of the bond between the two atoms. Equation (4.1) is Hooke's law, and the resulting motion is simple harmonic. Based on these assumptions the ball-and-spring model is a harmonic oscillator. The vibrational frequency ν_0, in Hz, of the harmonic oscillator in terms of classical mechanics is given by

$$\nu_0 = \frac{1}{2\pi}\sqrt{\frac{k}{m}} \tag{4.2}$$

where m is the reduced mass in kg:

$$m = \frac{m_1 m_2}{m_1 + m_2} \tag{4.3}$$

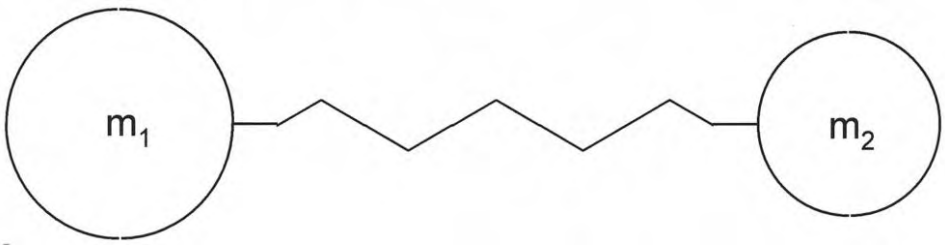

Figure 4.1. The ball and spring model for a diatomic molecule.

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In vibrational spectroscopy, it is common to use the wavenumber unit $\tilde{\nu}$, which is expressed in cm^{-1}. This is the number of waves in a length of one centimeter, the reciprocal wavelength, and is given by the following relationship:

$$\tilde{\nu} = \frac{1}{\lambda} = \frac{\nu}{c} \tag{4.4}$$

where λ is the wavelength and c is the velocity of light in vacuum (2.997925×10^8 m s^{-1}). The wavenumber unit has the advantage of being linear with energy.

Note that the only two quantities in Eq. (4.2) are the force constant and the reduced mass of the chemical bond. These two molecular properties determine the frequency at which a molecule will absorb infrared radiation. Thus, the vibrational frequency is higher when the force constant is higher, i.e., when the bonding between the two atoms is stronger. Conversely, the heavier the vibrating masses, the lower the frequency or wavenumber, respectively. To verify this and as a little OPUS exercise, you should load the files MIR\WATER and MIR\DWATER, which are the IR spectra of water and deuterium oxide, respectively. Using the crosshair cursor determine the wavenumber position of the peak height of the broad O–H and O–D stretching bands above 3000 cm^{-1} and 2000 cm^{-1}, respectively. The ratio of these two wavenumbers $\tilde{\nu}$ (O–H)/$\tilde{\nu}$ (O–D) is about 1.4 and almost the ratio

$$\sqrt{m_{O\text{–}D}/m_{O\text{–}H}} = \sqrt{34/18}.$$

According to quantum mechanics the vibrational energy of a harmonic oscillator E_{vib} is defined as follows:

$$E_{vib} = h\nu_0\left(n + \frac{1}{2}\right) = \frac{h}{2\pi}\sqrt{\frac{k}{m}}\left(n + \frac{1}{2}\right) \tag{4.5}$$

where h is Planck's constant (6.6256×10^{34} J s), k and m have the same definition as in Eq. (4.2). The vibrational quantum number n having the values of 0, 1, 2, 3 etc. characterizes the eigenstates of the harmonic oscillator. The energy of the ground state ($n = 0$), the so-called zero point energy, is given by

$$E_{vib,0} = \frac{1}{2}h\nu_0 \tag{4.6}$$

In our discussion so far, we have assumed that the motions of atoms in a vibrating molecule are harmonic. Although making this assumption made the mathematics easier, it is not a realistic view of the motion of atoms in a real vibrating molecule. Anharmonic motion is the type of motion that really takes place in vibrating molecules. The energy levels of such an anharmonic oscillator are approximately given by

$$E_{\text{vib}} = h\nu_0 \left[\left(n + \frac{1}{2} \right) - x_{\text{a}} \left(n + \frac{1}{2} \right)^2 \right] \tag{4.7}$$

where x_{a} is the anharmonicity constant.

The vibrations of a polyatomic molecule can be considered as a system of coupled anharmonic oscillators. If there are N atomic nuclei in the molecule, there will be a total of $3N$ degrees of freedom of motion for all the nuclear masses in the molecule. Subtracting the pure translations and rotations of the entire molecule leaves ($3N$-6) vibrational degrees of freedom for a non-linear molecule and ($3N$-5) vibrational degrees of freedom for a linear molecule. These internal degrees of freedom correspond to the number of independent normal modes of vibration. Note that in each normal mode of vibration all the atoms of the molecule vibrate with the same frequency and pass through their equilibrium positions simultaneously.

The determination of the form and of the frequency of normal modes of molecular vibrations is beyond the scope of this present book. The reader interested in this topic is referred to the relevant books listed in the bibliography.

4.2 The Infrared Spectrum

Infrared spectroscopy is based on the interaction of electromagnetic radiation with a molecular system, in most cases in the form of absorption of energy from the incident beam. The absorption of infrared light induces transitions between the vibrational energy levels given by Eq. (4.7). As shown in Fig. 4.2, the energy levels of the anharmonic oscillator are not equidistant.

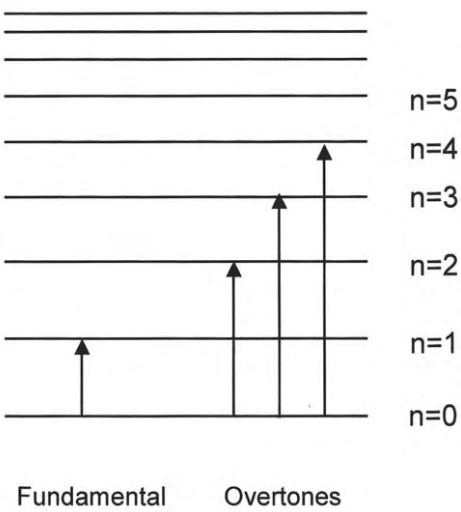

Figure 4.2. The energy levels of an anharmonic oscillator.

When a molecule is raised from the ground vibrational state ($n = 0$) to the first excited vibrational state ($n = 1$), it is said to undergo a fundamental transition. According to Eq. (4.7) the wavenumber of the fundamental transition is given by

$$\tilde{\nu}(0 \rightarrow 1) = \frac{\nu_0}{c}[1 - 2x_a] \tag{4.8}$$

The intensity of an infrared absorption band is proportional to the square of the change in the molecular electric dipole moment μ caused by a normal coordinate q:

$$I_{IR} \propto \left(\frac{\partial \mu}{\partial q}\right)^2 \tag{4.9}$$

In other words a normal mode is infrared active, if this mode alters the dipole moment of the molecule and thus fulfils the requirement

$$\frac{\partial \alpha}{\partial q} \neq 0 \tag{4.10}$$

The vast majority of molecules have infrared bands in the spectral range between 400 and 4000 cm^{-1}. Most of the intense features in any mid-infrared spectrum can be assigned to fundamental transitions.

As a consequence of anharmonicity, transitions from the ground state to higher excited states ($n = 2,3,4...$) are also allowed. This type of transition is called an overtone transition. For example, the overtone transition from $n = 0$ to $n = 2$ appears at a wavenumber of

$$\tilde{\nu}(0 \rightarrow 2) = 2\frac{\nu_0}{c}[1 - 3x_a] \tag{4.11}$$

Overtones can be recognized because they give rise to very weak absorption bands, often at about twice the wavenumber of a fundamental transition.

4.3 The Raman Spectrum

Raman spectroscopy is based on inelastic scattering of light by matter. The simplest way of explaining the classical or spontaneous Raman effect is via an energy level diagram such as that depicted in Fig. 4.3. Let us assume that the molecular system has two vibrational energy levels, the ground state $n = 0$ and the excited state $n = 1$, which are separated by the energy $h\nu_M$, where ν_M is the frequency of the molecular vibration. The incident light with energy $h\nu_L$ induces transitions to virtual levels as shown. Returning to the initial state takes place in three different ways, namely by emitting light of frequencies ν_L, $\nu_L - \nu_M$, and $\nu_L + \nu_M$. The elastic or Rayleigh scattering arises from a transition that starts and finishes at the same vibrational energy level. The shifts to lower

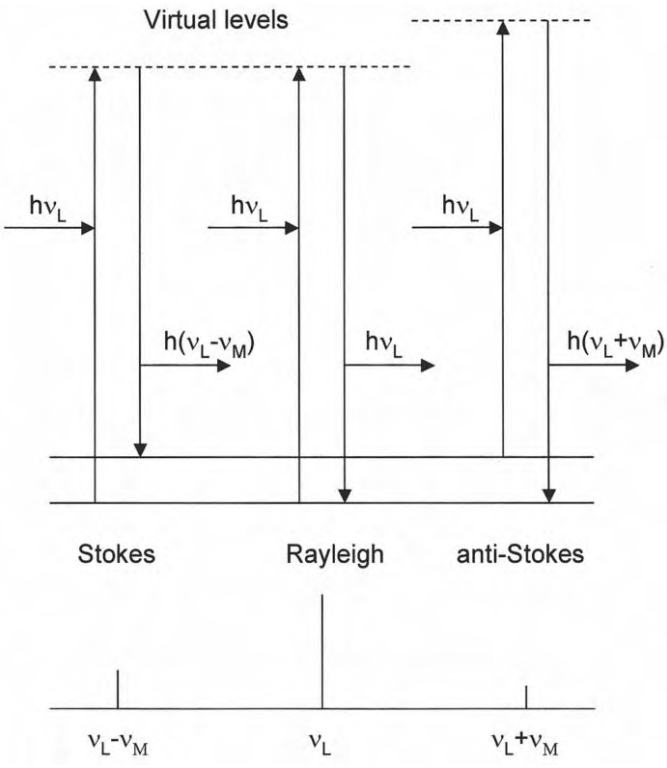

Figure 4.3. The energy level diagram showing the basic transitions involved in the spontaneous Raman scattering. The two-level system is excited by the energy of $h\nu_L$.

and higher frequencies are known as Stokes and anti-Stokes Raman scattering, respectively. Stokes Raman scattering arises from a transition that starts at the ground state vibrational energy level and finishes at a higher vibrational energy level, whereas anti-Stokes Raman scattering involves a transition from a higher to a lower vibrational energy level. At ambient temperatures, most molecular vibrations are in the ground state and thus the anti-Stokes transitions are less likely to occur than the Stokes transitions, resulting in the Stokes Raman scattering being more intense. For this reason, it is usually the Stokes Raman spectrum that is routinely studied.

OPUS differentiates between the Raman spectrum and the single channel spectrum. The single channel spectrum of the Stokes and anti-Stokes Raman scattering for a sulphur sample is shown in Fig. 4.4. Note that the abscissa here is expressed in absolute wavenumbers. Therefore, the exciting laser line appears at $\tilde{\nu}_L = 9394\ \text{cm}^{-1}$ and the bands at wavenumbers lower and higher than $9394\ \text{cm}^{-1}$ arise from Stokes and anti-Stokes Raman scattering, respectively. On the other hand, a standard Raman spectrum comprises the spectral range from 0 to $3500\ \text{cm}^{-1}$. Load the file RAMAN\SULPHUR and find out the difference between these two types of spectra.

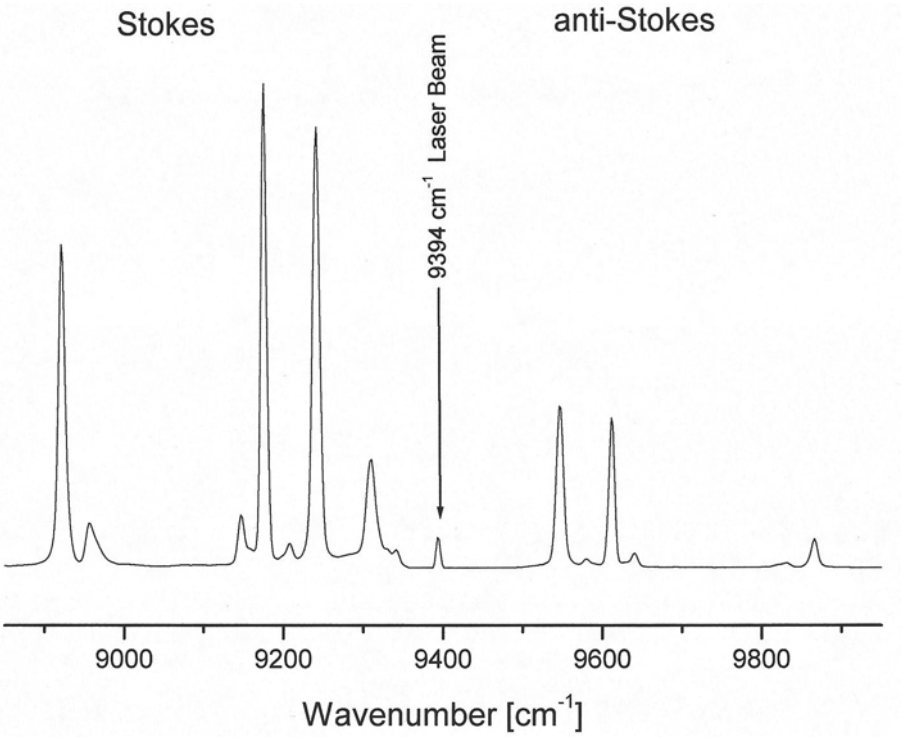

Figure 4.4. The Stokes and anti-Stokes Raman spectrum of sulphur at 1064 nm laser excitation. The feature between 9300 and 9394 cm^{-1} is an experimental artefact due to the optical filter used to suppress the very intense Rayleigh band.

The intensity of Raman scattering is proportional to the square of the change in the molecular polarizability α resulting from a normal mode q:

$$I_{RA} \propto \left(\frac{\partial \alpha}{\partial q}\right)^2 \tag{4.12}$$

Otherwise stated, a vibrational mode that satisfies the requirement

$$\frac{\partial \alpha}{\partial q} \neq 0 \tag{4.13}$$

is said to be Raman active.

One should be aware of the fact that Raman spectroscopy is a complementary technique to IR spectroscopy. In cases where a chemical compound exhibits a centre of symmetry, certain normal vibrations will be only Raman active and certain normal vibrations will be only IR active. Thus, one needs both techniques to

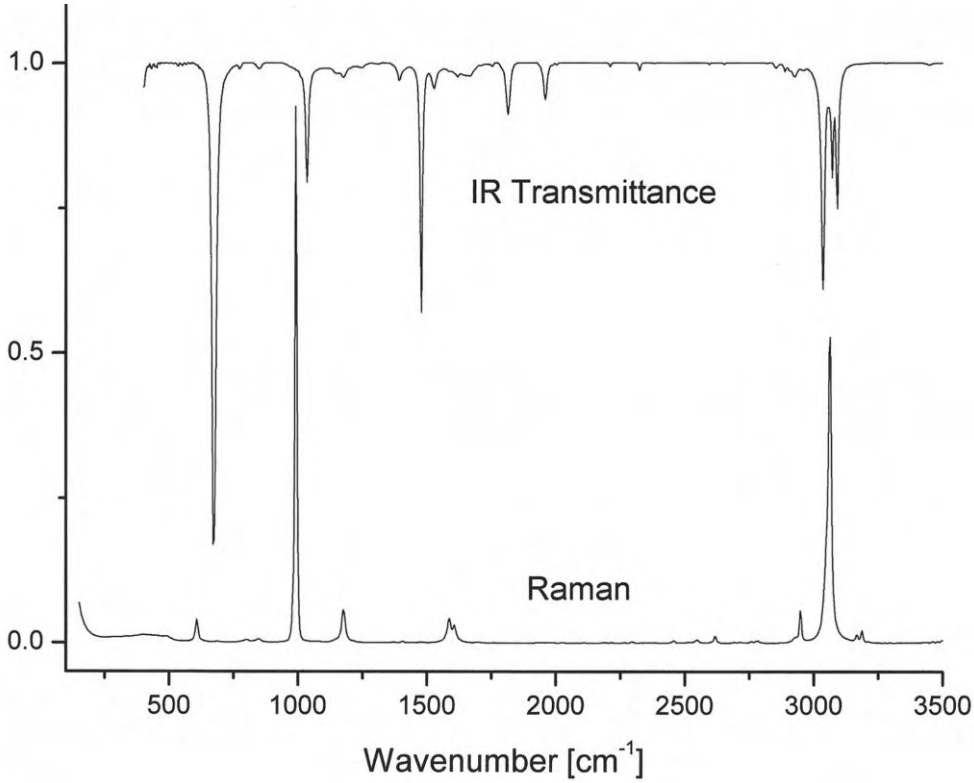

Figure 4.5. Comparison of the IR and Raman spectra of benzene. Top: IR transmittance spectrum; bottom: Raman spectrum.

record the complete vibrational spectrum of a substance. Moreover, bands that are strong in the Raman are usually weak in the IR, and vice versa in cases where the normal modes are allowed in both techniques. Generally, strong IR bands are related to polar functional groups, whereas non-polar functional groups give rise to strong Raman bands. As an example, Fig. 4.5 clearly shows the difference between the IR and Raman spectrum of benzene.

5 Fourier Transform Technique

5.1 The Michelson Interferometer

The heart of the optical hardware in a FT spectrometer is the interferometer. Nowadays, the most common set-up used is the classic two-beam Michelson interferometer shown schematically in Fig. 5.1. It consists of two mutually perpendicular plane mirrors, a fixed mirror M1 and a movable one M2. A semi-reflecting mirror, the beam splitter, bisects the planes of these two mirrors. A beam emitted by a source S is split in two by the beam splitter. The reflected part of the beam travels to the fixed mirror M1 through the distance L, is reflected there and hits the beam splitter again after the total path length of $2L$. The same happens to the transmitted radiation. However, as the mirror M2 is not fixed at the same position L but can be moved very precisely back and forth around L by a distance x, the total path length of the transmitted part is accord-

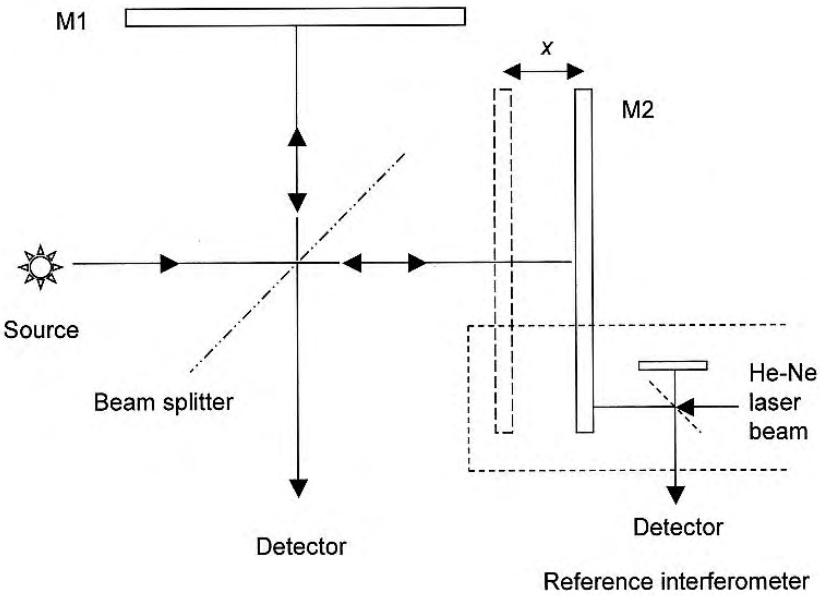

Figure 5.1. The schematic of a Michelson interferometer.

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ingly $2(L + x)$. Thus, when the two parts of the beam recombine at the beam splitter they possess an optical path length difference of $\delta = 2x$. Since the two split beams are spatially coherent, they interfere on recombination. Naturally, only half of the radiation entering the interferometer can get out in the out direction; half is reflected back towards the input.

In the case of a *monochromatic* ray, the two bundles of radiation interfere constructively if their optical retardation is a multiple of the wavelength λ, i.e.

$$\text{if } \delta = 2x = n\lambda \quad \text{with} \quad n = 0,1,2,3.... \tag{5.1}$$

and destructively if δ is an odd multiple of $\lambda/2$, i.e.

$$\text{if } \delta = 2x = (2n + 1)\frac{\lambda}{2} \tag{5.2}$$

The beam, modulated by the movement of the mirror M2, leaves the interferometer and is finally focussed on the detector. The signal actually registered by the detector is thus the radiation intensity of the combined beams as a function of the retardation δ. For a source of monochromatic radiation with frequency ν (or wavenumber $\tilde{\nu} = 1/\lambda = \nu/c$), the intensity at the detector is given by the equation

$$I_{\mathrm{D}}(\delta) = 0.5S(\tilde{\nu})[1 + \cos(2\pi\tilde{\nu}\delta)] \tag{5.3}$$

where $S(\tilde{\nu})$ is the intensity of the monochromatic beam. It can be seen that $I_{\mathrm{D}}(\delta)$ is composed of a constant (dc) component and a modulated (ac) component. Only the ac component is important in spectroscopic measurements, and it is this modulated component that is generally referred to as the interferogram:

$$I(\delta) = S(\tilde{\nu})\cos(2\pi\tilde{\nu}\delta) \tag{5.4}$$

As the retardation is a function of time, the interferogram is a function defined in the time domain. If the movable mirror is scanned at a constant velocity v, the retardation is simply

$$\delta = 2vt \tag{5.5}$$

and the interferogram $I(t)$ is given by

$$I(t) = S(\tilde{\nu})\cos(2\pi\tilde{\nu}2vt) \tag{5.6}$$

Therefore, the signal at the detector varies sinusoidally with the frequency

$$f_{\tilde{\nu}} = 2v\tilde{\nu} \tag{5.7}$$

For a source of *polychromatic* radiation the interference pattern is given by

$$I(\delta) = \int_0^\infty S(\tilde{\nu})\cos(2\pi\tilde{\nu}\delta)\mathrm{d}\tilde{\nu} \tag{5.8}$$

where $S(\tilde{\nu})$ is now the spectral power density of the source. Note one feature: at zero optical path length difference ($\delta = 0$) all the radiation of whatever frequency passes through the interferometer. At any other path difference, only some will pass. Hence, an interferogram is characterised by a "centre burst" at zero path difference and a very complex pattern of waves symmetrically dispersed about it.

Generally, it is difficult to understand an interferogram, because we are used to thinking in the frequency (or wavenumber) domain, namely in terms of spectra $S(\tilde{\nu})$. As an illustration a few simple spectra and their interferograms are depicted in Fig. 5.2. Figure 5.2a represents the simple case when two closely spaced lines of unequal intensity are examined. In Fig. 5.2b and c both bands have Gaussian profiles and yield sinusoidal interferograms with a Gaussian envelope. As can be seen, the narrower the width of the spectral band the greater the width of the envelope of the interferogram.

The mathematics of the conversion of an interferogram into a spectrum is the Fourier transformation (FT). Accordingly, the spectrum is given by

$$S(\tilde{\nu}) = \int_{-\infty}^{+\infty} I(\delta)\cos(2\pi\tilde{\nu}\delta)\mathrm{d}\delta \tag{5.9}$$

Equations (5.8) and (5.9) are interconvertible and are known as a Fourier transform pair.

Do not worry if your knowledge of calculus is not up to these equations. Fortunately, it is not necessary to have a detailed knowledge of the mathematics involved in order to carry out experiments using an FT spectrometer. Based on fully developed software the computer performs these transformations.

The essential steps for obtaining an FT-IR spectrum are to produce an interferogram with and without a sample in the beam and then transform these interferograms into spectra of the source with sample absorption and the source without sample absorption (see for instance MIR files GLYCIN, WATER, and VASELINE). The ratio of the former and the latter is the IR transmission spectrum of the sample.

In the case of FT-IR spectroscopy, the sample is usually placed between the interferometer and the detector. For FT Raman spectroscopy the scattering volume of the sample itself is the radiation source.

5.2 Advantages of Fourier Transform Spectroscopy

5.2.1 Connes Advantage

Equation (5.4) is extremely useful for practical measurements, because it allows a very precise tracking of the moveable mirror. In fact, all modern FT-IR and FT Raman spectrometers use the interference pattern of the monochromatic light of a He–Ne laser ($\lambda_{\text{He–Ne}} = 633$ nm or $\tilde{\nu} = 15800$ cm^{-1}) in order to control the change in optical path length difference. To emphasize this the reference inter-

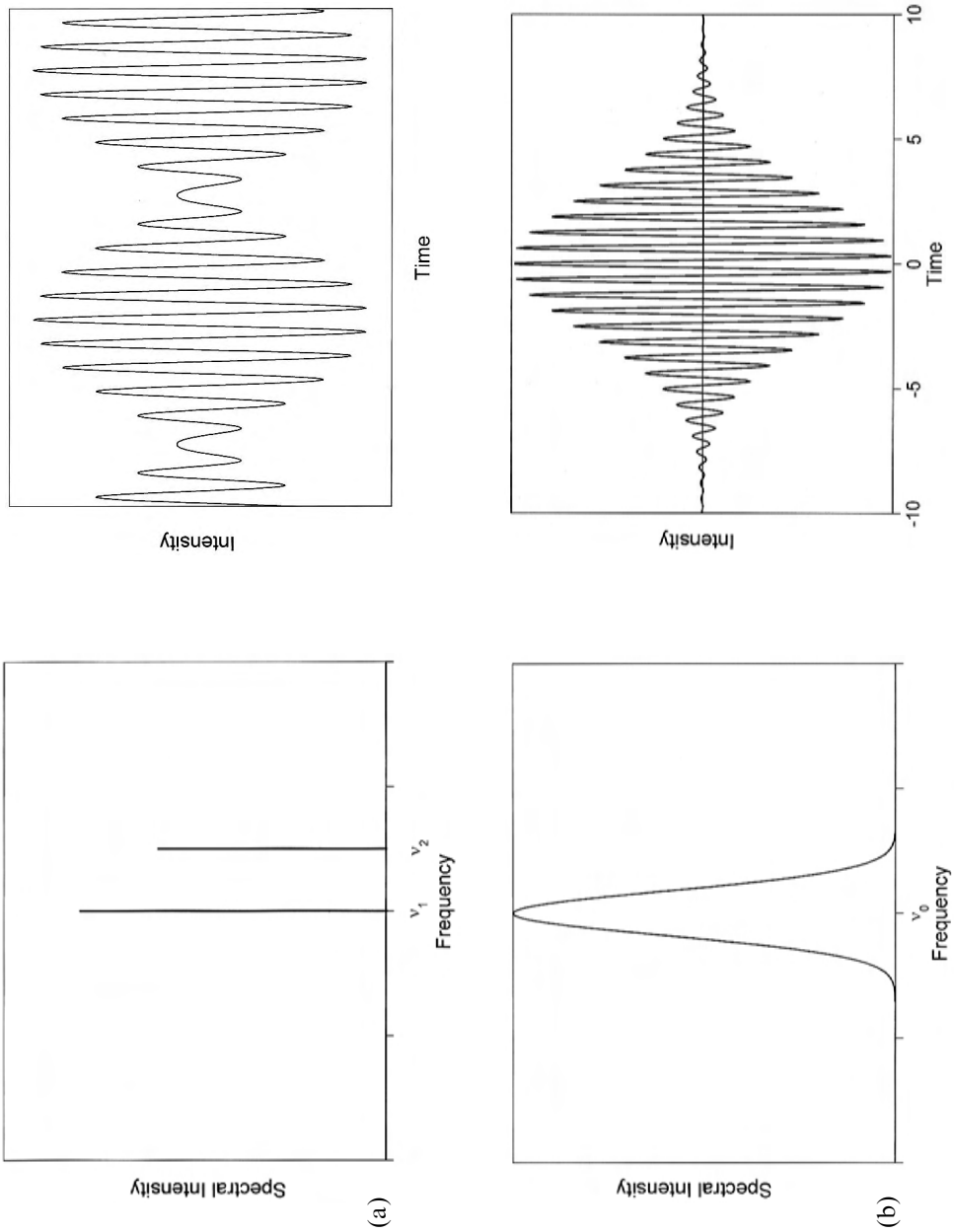


Figure 5.2. (cont. on page 39)

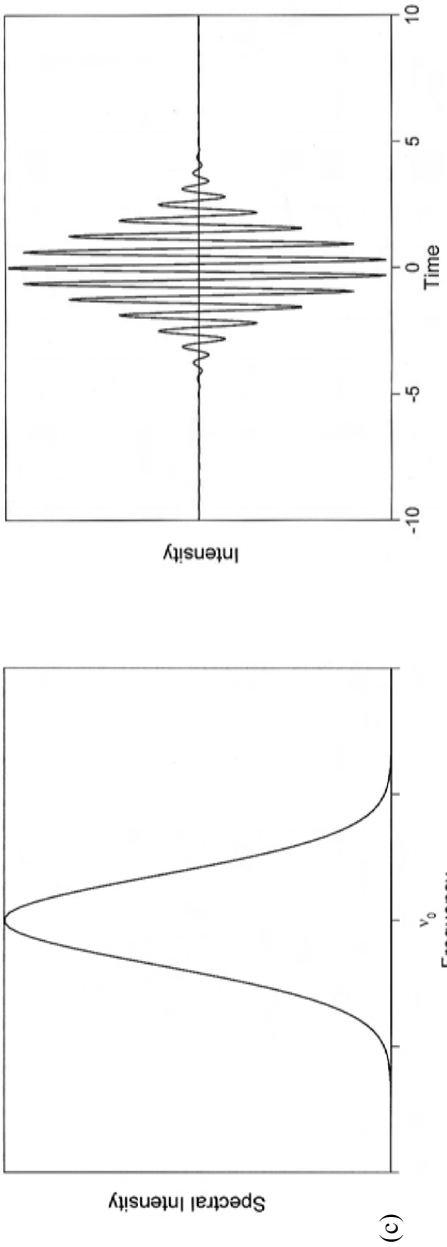


Figure 5.2. Examples of spectra (left) and their corresponding interferograms (right): (a) two monochromatic lines of unequal intensity; (b) small Gaussian line; (c) broad Gaussian line.

ferometer is included in Fig. 5.1. The interferogram is digitised precisely at the zero crossing points of the laser interference pattern. As a zero crossing occurs every $\lambda/2$, the minimum possible sampling spacing $\Delta x_{\min}$ is 1/31600 cm or 0.31645 µm. Obviously, the accuracy of the sample spacing Δx between two zero crossings is solely determined by the precision of the laser wavelength itself. As the sample spacing $\Delta\tilde{\nu}$ in the spectrum is inversely proportional to Δx, the error in $\Delta\tilde{\nu}$ is of the same order as in Δx. Hence, FT-IR spectrometers have a built-in wavenumber calibration of high precision (practically about 0.01 cm^{-1}). This advantage is known as the Connes advantage.

5.2.2 Jacquinot Advantage

The so-called Jacquinot or throughput advantage arises from the fact that the circular aperture used in FT-IR spectrometers exhibits a larger area than the linear slits used in grating spectrometers, thus enabling a higher throughput of radiation.

5.2.3 Fellget Advantage

In conventional spectrometers, the spectrum $S(\tilde{\nu})$ is measured directly by recording the intensity at different monochromator settings $\tilde{\nu}$, one $\tilde{\nu}$ after the other. In an FT spectrometer, all frequencies emanating from the source impinge simultaneously on the detector. This accounts for the so-called multiplex- or Fellget advantage.

The measuring time in an FT spectrometer is the time needed to move the mirror M2 over a distance proportional to the desired resolution. As the mirror can be moved very fast, completed spectra can be measured in fractions of a second.

Details about the advantages of the FT technique can be found in the literature [Griffith and de Haseth; Kauppinen and Partanen].

5.3 Discrete Fourier Transformation

As already mentioned above, in a laser-controlled FT spectrometer, the interferogram is digitised and consists of N discrete, equidistant points. That means that instead of Eq. (5.9) we have to use the discrete Fourier transformation:

$$S(k\Delta\tilde{\nu}) = \sum_{n=0}^{N-1} I(n\Delta x)\exp(2\pi i kn/N) \tag{5.10}$$

where the continuous variables δ and $\tilde{\nu}$ have been replaced by $n\Delta x$ and $k\Delta\tilde{\nu}$, respectively. Alternatively, if the set $S(k\Delta\tilde{\nu})$ of Fourier coefficients is known, the computer can easily reconstruct the interferogram by the inverse discrete Fourier transformation

$$I(n\Delta x) = (1/N)\sum_{k=0}^{N-1} S(k\Delta\tilde{\nu})\exp(-2\pi i nk/N) \tag{5.11}$$

The spacing $\Delta\tilde{\nu}$ in the spectrum is related to Δx by

$$\Delta\tilde{\nu} = \frac{1}{N\Delta x} \tag{5.12}$$

In practice, Eq. (5.10) is seldom used directly because it is highly redundant. Instead most manufacturers apply the so-called fast Fourier transform (FFT) algorithm, preferably according to Cooley and Tukey [1]. The aim of this FTT is to reduce the number of complex multiplications and sine- and cosine calculations appreciable, leading to a substantial saving of computer time. The price paid for the speed is that the number of interferogram points N cannot be chosen at will, namely N must be a power of two. For this reason, it follows that the spectra recorded with laser-controlled FT spectrometers exhibit a sampling spacing of

$$\Delta\tilde{\nu} = \frac{m}{2^N}\tilde{\nu}_{\text{laser}} \tag{5.13}$$

The use of the discrete FT can lead to spectral artefacts: the *picket fence effect* and *aliasing*.

5.3.1 Picket Fence Effect: Zerofilling

The picket fence effect becomes evident when the interferogram contains frequencies that do not coincide with the frequency sample points. If, in the worst case, a frequency component lies exactly halfway between two sample points, an erroneous signal reduction by 36% can occur. In other words, one seems to be viewing the true spectrum through a picket fence, thereby clipping those spectral parts lying "behind the pickets", i.e. between the sampling positions (see file APODIZ\BoxcarZF2). The picket fence effect can be overcome by adding zeros to the end of the interferogram before the discrete FT is performed, thereby increasing the number of points per wavenumber in the spectrum (see files APODIZ\BoxcarZF4 and ZF8). Hence, zerofilling the spectrum is a form of interpolating the spectrum, reducing the error. As a rule of thumb, one should always at least double the original interferogram size for practical measurements by zerofilling it, i.e. one should choose a zerofilling factor of two. In those cases, however, where the expected line width is similar to the spectral sample spacing (as e.g. in the case of gas phase spectra) a zerofilling factor of up to 8 may be appropriate. It should be noted that zerofilling does not introduce any errors, because the instrumental line shape is not changed by this procedure.

5.3.2 Aliasing

Another possible artefact due to the use of the discrete FT is the endless replication of the original spectrum and its mirror image on the wavenumber axis, which is termed aliasing. In order to avoid aliasing the Nyquist criterion must be fulfilled. This criterion states that any waveform that is a sinusoidal function of time can be sampled unambiguously using a sampling frequency greater than

or equal to twice the bandwidth of the system. For details concerning aliasing see Griffith and de Haseth.

An advanced FT software package will automatically account for proper sampling, such that only the upper and lower limits of the desired spectral range need to be specified by the user.

5.4 Effect of the Finite Record Length: Leakage and Apodization

Equation (5.9) shows that in order to measure the complete spectrum, we would have to scan the moving mirror of the interferometer an infinitely long distance, with δ varying between $-\infty$ and $+\infty$ centimeters. In practice, the optical path length difference is finite. By restricting the maximum retardation to l, we are effectively multiplying the complete interferogram by the boxcar truncation function (see Fig. 5.3a left)

$$D(\delta) = 1 \qquad \text{if } -l \leq \delta \leq +l \tag{5.14}$$
$$D(\delta) = 0 \qquad \text{if } \delta > |l|$$

The spectrum in this case is given by:

$$S_l(\tilde{\nu}) = \int_{-\infty}^{\infty} I(\delta)D(\delta)\cos(2\pi\tilde{\nu}\delta)\mathrm{d}\delta \tag{5.15}$$

According to the convolution theorem of Fourier analysis, the Fourier transform of a product of two functions is given by the convolution (here indicated by the symbol '$\otimes$') of their individual Fourier transforms. Hence, the effect of multiplying $I(\delta)$ by the boxcar function $D(\delta)$ is to yield a spectrum that is the convolution of the Fourier transform of $I(\delta)$ measured with an infinitely long retardation and the Fourier transform of $D(\delta)$. The Fourier transform of $I(\delta)$ is the true spectrum $S(\tilde{\nu})$, while the Fourier transform of $D(\delta)$, $f(\tilde{\nu})$, is given by

$$f(\tilde{\nu}) = \frac{2l\sin(2\pi\tilde{\nu}l)}{2\pi\tilde{\nu}l} \tag{5.16}$$

Therefore, the spectrum $S_l(\tilde{\nu})$ measured with a finite length retardation is described by

$$S_l(\tilde{\nu}) = S(\tilde{\nu}) \otimes f(\tilde{\nu}) \tag{5.17}$$

The result is that the Fourier transform of a monochromatic source is not an infinitely narrow line, but has the shape of the $(\sin x)/x$ function. As shown in Fig. 5.3a right, this function is centred about $\tilde{\nu} = 0$ and intersects the $\tilde{\nu}$ axis at $\tilde{\nu} = n/2l$, where $n = 1, 2, 3,\ldots$, so that the first intersection occurs at a wavenumber $1/2l$. Obviously, the main maximum at $\tilde{\nu} = 0$ has a series of negative and positive side lobes or 'feet' with diminishing amplitudes. These side lobes cause a 'leakage' of the spectral intensity, *i.e.* the intensity is not strictly

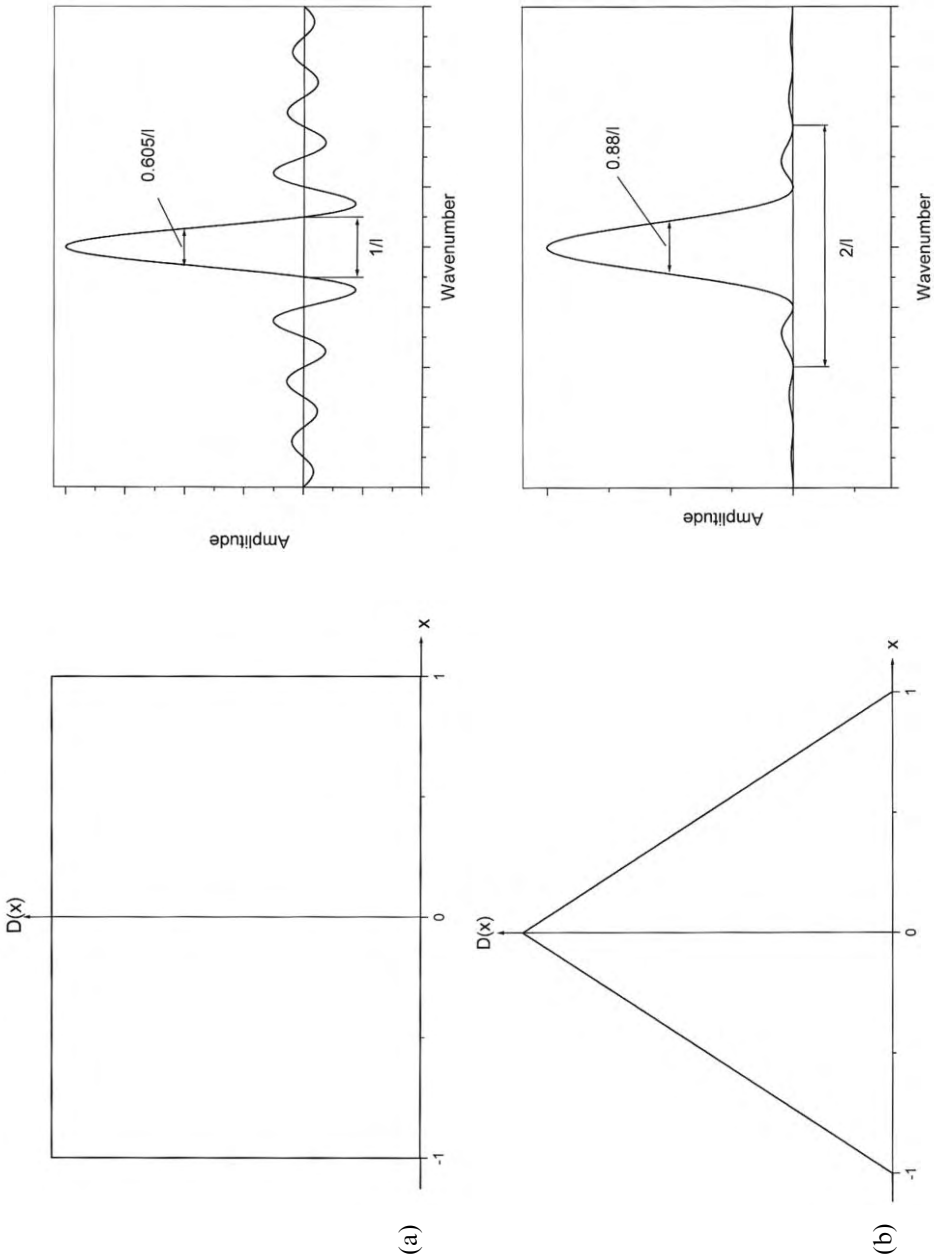
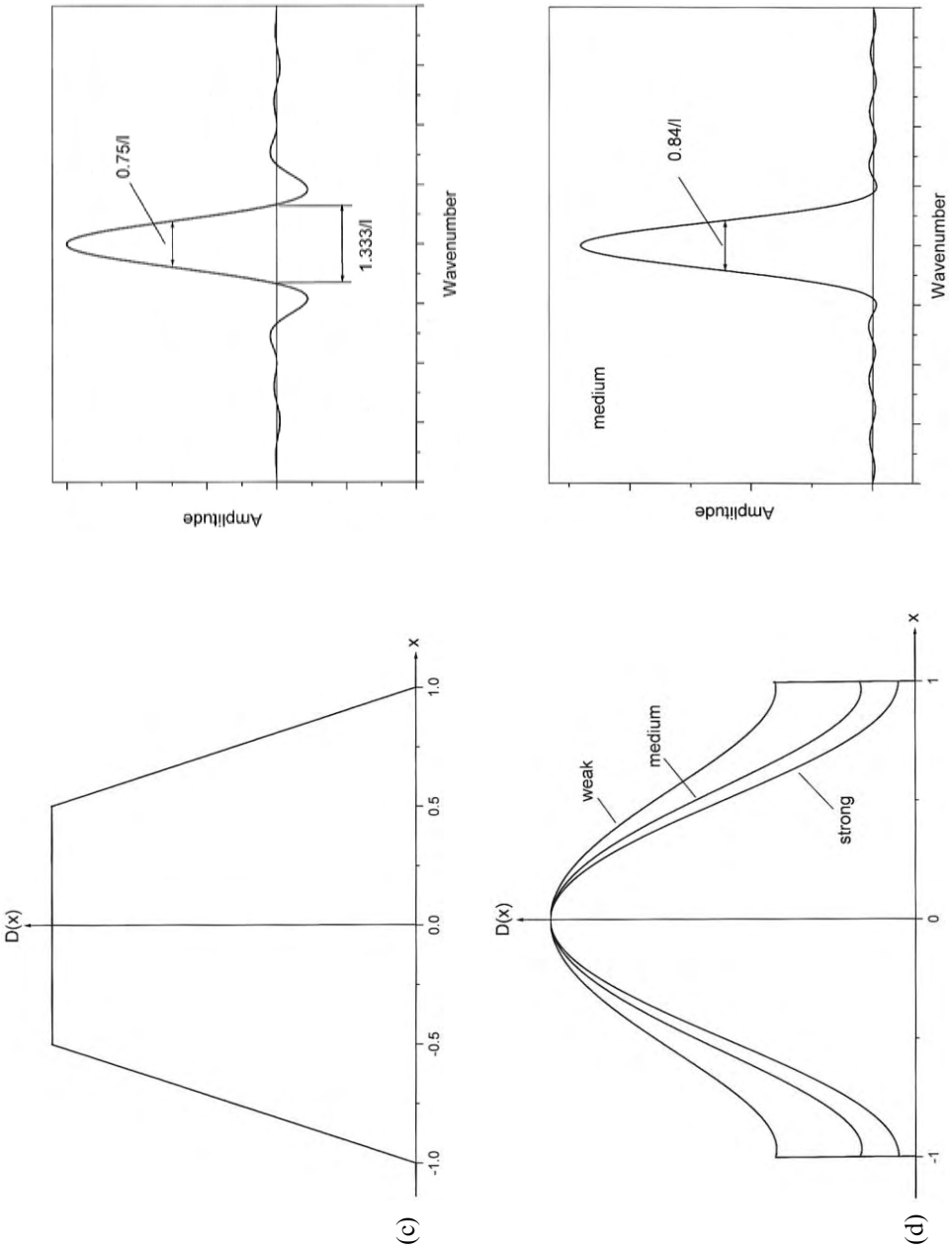


Figure 5.3. (cont. on page 44–45)


D(x)
x
-1.0
-0.5
0.0
0.5
1.0
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Amplitude
0.75/l
1.333/l
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x
-1
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1
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medium
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Amplitude
0.84/l
medium
Wavenumber

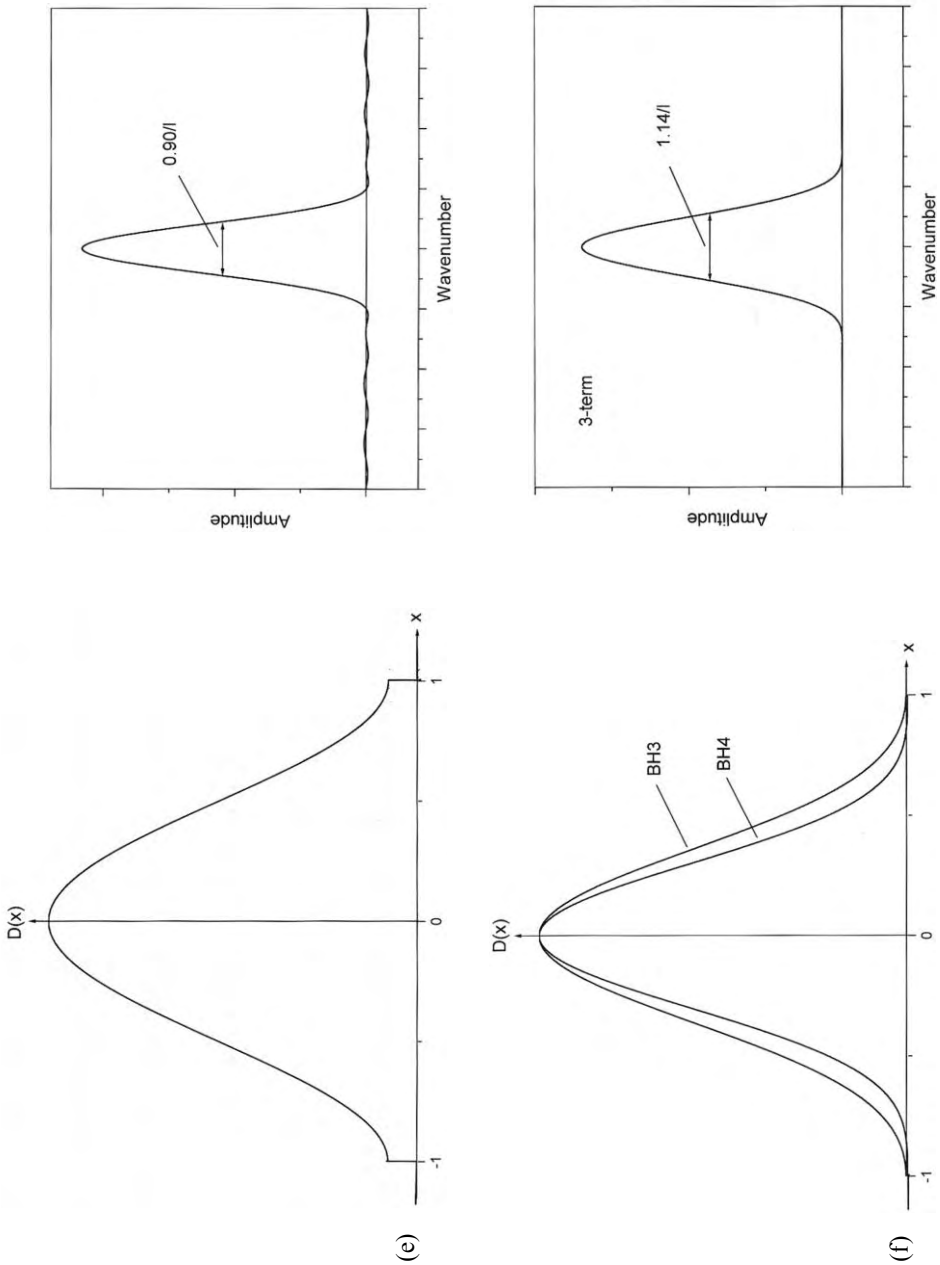

Figure 5.3. Various apodization functions (left) and the 'instrumental lineshape' produced by them (right): (a) boxcar truncation; (b) triangular; (c) trapezoidal; (d) Norton–Beer weak, medium, and strong; (e) Happ–Genzel; (f) Blackman–Harris 3-term and 4-term. The maximum retardation is set to $l = 1$. In the Fourier transform the FWHH of the main lobe is indicated.

localized but contributes also to these side lobes. So, the largest side lobe amplitude is 22% of the main lobe amplitude.

As the side lobes do not correspond to actually measured information but rather represent an artefact due to the abrupt truncation, it is desirable to reduce their amplitude. The process that attenuates the spurious feet in the spectral domain is known as apodization (originating from the Greek word "αποδοσ", which means 'without feet'). In other words, apodization is the removal of the side lobes by multiplying the interferogram by a suitable function before the Fourier transformation is carried out. The price paid for suppressing the side lobes is that one has to accept a broadening of spectral lines.

When a spectrum is measured on a dispersive instrument, the true spectrum is convolved with the instrumental line shape (ILS) of the monochromator, which is the triangular slit function. The situation with the FT technique is equivalent, except that the true spectrum is convolved with the (sinx)/x function (no apodization) or with the FT of an appropriate apodization function. Hence, FT instruments offer a free choice of ILS according to the apodization selected and thus make it possible to optimise the sampling condition for a particular application.

The FWHH of the ILS defines the best resolution achievable with a given apodization function. This is because if two spectral lines are to appear resolved from each other, they must be separated by at least the distance of their FWHH, otherwise no 'dip' will occur between them. As suppression of a side lobe always causes broadening of the main lobe, leakage reduction is only possible at the cost of resolution.

In the OPUS software you can choose between apodization functions as follows:

Triangular

$$D_{\mathrm{TR}}(x) = 1 - \frac{|x|}{l} \qquad \text{for } |x| \leq l \tag{5.18}$$

$$D_{\mathrm{TR}} = 0 \qquad \text{for } |x| > l$$

Trapezoidal or four points

$$D_{\mathrm{FP}} = 1 \qquad \text{for } |x| \leq l/2$$

$$D_{\mathrm{FP}} = 2 - \frac{2|x|}{l} \qquad \text{for } l/2 < |x| \leq l \tag{5.19}$$

$$D_{\mathrm{FP}} = 0 \qquad \text{for } |x| > l$$

Norton–Beer

$$D_{\mathrm{NB}}(x) = \sum_{i=0}^{3} C_i \left[1 - \left(\frac{x}{l} \right)^2 \right]^i \qquad \text{for } |x| \leq l \tag{5.20}$$

$$D_{\mathrm{NB}} = 0 \qquad \text{for } |x| > l$$

Table 5.1. Coefficients of the Norton–Beer apodization functions.

Function	C_0	C_1	C_2	C_3
Weak	0.384093	–0.087577	0.703484	0
Medium	0.152442	–0.136176	0.983734	0
Strong	0.045335	0	0.554883	0.399782

The coefficients C_i for each are shown in Tab. 5.1. Obviously, the boxcar truncation is a particular case of Eq. (5.20), i.e. $i = 0$; $C_0 = 1$

Blackman–Harris

$$D_{\mathrm{BH}} = \sum_{i=1}^{3} A_i \cos(i\pi \frac{x}{l}) \qquad \text{for } |x| \leq l \tag{5.21}$$

$$D_{\mathrm{BH}} = 0 \qquad \text{for } |x| > l$$

The coefficient A_i of the three functions *Happ–Genzel, Blackman–Harris* three term, and *Blackman–Harris* four term are summarized in Tab. 5.2.

All these apodization functions are plotted in Fig. 5.3 left. Their corresponding Fourier transforms are presented in Fig. 5.3 right, together with the FWHH of the main lobe. As expected, we see that due to apodization the side lobes are suppressed and the main lobes are broader than that of the $(\sin x)/x$ function.

The choice of a particular apodization depends on what one is aiming at. If the optimum resolution of $0.605/l$ is mandatory, the boxcar truncation (no apodization at all) should be chosen. If a loss of resolution of 50% compared to the boxcar can be tolerated, the Happ–Genzel, or even better, the Blackman–Harris three term apodization is recommended. Since the Blackman–Harris window shows the highest side lobe suppression and is furthermore nearly zero at the interval ends, it can be considered the top performer.

To verifying how the choice of apodization option affects the spectral features load the files in the folder APODIZ. Here you will find FT Raman spectra of cyclohexane, each of them were acquired with 64 scans at a resolution of 4 cm^{-1} and a zerofilling factor of 2, but with various apodization functions.

Table 5.2. Coefficients of the Happ–Genzel and Blackman–Harris apodization functions.

Function	A_0	A_1	A_2	A_3
Happ–Genzel	0.54000	0.46000	0	0
BH 3-term	0.42323	0.49755	0.07922	0
BH 4-term	0.35875	0.48829	0.14128	0.01168

Cyclohexane shows a rather narrow Raman band at 801 cm^{-1} due to the ring-breathing mode. As mentioned in Section 3.5, you can select the relevant spectral range by right-clicking at any point of the spectrum window, which open the pop-up menu to access the spectrum properties. There are two possibilities: Either use the *Zoom* button → *Zoom in* to draw a frame around the band at 801 cm^{-1} or click on *Properties* and insert the values of the spectral range wanted, e.g. 780–830 cm^{-1}. For the case of the boxcar function, you will find that beside the main band (FWHH ~ 4 cm^{-1}) there are several side lobes. Of course, the band is additionally distorted due to the digitalisation. Comparing the files, it is obvious that apodization causes a suppression of the side lobes as well as a decrease in the peak height and a broadening of the band. So, for the case of Blackman–Harris four term the FWHH amounts to almost 8 cm^{-1}. For a more illustrative comparison, it is useful to normalize the band of the various files by clicking the button *Scale all Spectra* → *Maximize each spectrum (Y)* of the pop-up menu.

Another point of interest is to examine how the choice of the zerofilling factor affects the spectral feature. For these purposes load additionally the files BoxcarZF4 and ZF8, which are again FT Raman spectra of cyclohexane taken with the boxcar truncation and the zerofilling factors 4 and 8, respectively. From the band at 801 cm^{-1}, it can be seen that the zerofilling factor of 8 produces a band shape resembling the theoretical curve shown in Fig. 5.3a right. Compare this band also with that acquired with Blackman–Harris four term and zerofilling factors of 2 and 8, respectively (files BH4T and BH4TZF8).

5.5 Phase Correction

In practice, the interferogram measured is not mirror symmetrical about the point $\delta = 0$. Call up the interferogram of the file MIR\GLYCIN or ACQUIS in order to verify that. This asymmetry originates from experimental errors, e.g., wavenumber-dependent phase delays of the optics, the detector/ amplifier unit, or the electronic filters. The Fourier transformation of such an asymmetrical interferogram generally yields a complex spectrum $C(\tilde{\nu})$ rather than a real spectrum $S(\tilde{\nu})$ as known from spectrometers based on the dispersive technique. That is why phase correction is necessary.

A complex spectrum $C(\tilde{\nu})$ can be represented by the sum

$$C(\tilde{\nu}) = R(\tilde{\nu}) + \mathrm{i}I(\tilde{\nu}) \tag{5.22}$$

of a purely real part $R(\tilde{\nu})$ and a purely imaginary part $I(\tilde{\nu})$, or, equivalently, by the product

$$C(\tilde{\nu}) = S(\tilde{\nu})\exp(\mathrm{i}\Phi(\tilde{\nu})) \tag{5.23}$$

of the true 'amplitude' spectrum $S(\tilde{\nu})$ and the complex exponential function $\exp(\mathrm{i}\Phi(\tilde{\nu}))$ containing the wavenumber-dependent 'phase' $\Phi(\tilde{\nu})$.

The purpose of the phase correction procedure is to determine the amplitude spectrum $S(\tilde{\nu})$ from the complex output $C(\tilde{\nu})$ of the FT of the interferogram. This can be performed either by calculating the square root of the 'power' spectrum

$$S(\tilde{\nu}) = \sqrt{R^2(\tilde{\nu}) + I^2(\tilde{\nu})} \tag{5.24}$$

or by multiplication of $C(\tilde{\nu})$ by the inverse of the phase exponential and taking the real part of the results:

$$S(\tilde{\nu}) = \mathrm{Re}\{C(\tilde{\nu})\exp(-\mathrm{i}\Phi(\tilde{\nu})\} \tag{5.25}$$

where the phase $\Phi(\tilde{\nu})$ is given by

$$\Phi(\tilde{\nu}) = \arctan[I(\tilde{\nu})/R(\tilde{\nu})] \tag{5.26}$$

Equations (5.24) and (5.25) are equivalent, if one deals with perfect data free of noise. However, if noise is present, as is always the case with measured data, noise contributions computed from Eq. (5.24) are always positive and in the worst case, a factor of $\sqrt{2}$ larger than the corrected signed noise amplitude computed from Eq. (5.25). The procedure according to Eq. (5.24) and (5.25) is known as the *power* and *Mertz* method, respectively.

5.6 Acquisition

To record a spectrum one has also to specify the manner in which the moving mirror in the interferometer should operate. Generally, there are acquisition modes as follows:

- *Single Sided*

This option allows only one-sided interferograms to be measured. Data are acquired during the forward movement of the mirror.

- *Double Sided*

The interferogram is measured on both sides. Data are acquired during the forward movement of the mirror. This mode yields an improvement in the signal-to-noise ratio as compared to the *Single Sided* mode. The resolution in time however amounts to half the value achieved in *Single Sided* mode.

- *Forward/Backward*

Data are collected during the forward and backward scan of the mirror. Forward and backward scans are coadded separately, calculated and then added. This mode has less dead time and a better signal-to-noise ratio, but requires twice as much computation time.

- *Fast Return*

This mode offers a fast mirror retrace without data collection. There is less dead time and a better signal-to-noise ratio.

For the same acquisition time the signal-to-noise ratio improves in the following order:

S/N (no *Fast Return*) < S/N *(Fast Return*) < S/N *(Forward/Backward*).

Double Sided acquisition is the method of choice for Raman experiments and precise quantitative measurements.

To verify the properties of the various acquisition modes call up the files in the folder ACQUIS. These are Raman spectra and interferograms of cyclohexane recorded with different acquisition versions. Find out for yourself which instrumental parameters have been used to record the spectra. Which spectra exhibit artifacts?

5.7 Raman Spectroscopy: Interferometer versus Grating Technique

Unlike IR spectroscopy where nowadays FT instrumentation is solely used, in Raman spectroscopy both conventional dispersive and FT techniques have their applications, the choice being governed by several factors. The two techniques differ significantly in several performance criteria, and neither one is 'best' for all applications. Contemporary dispersive Raman spectrometers are often equipped with silicon-based charge coupled device (CCD) multichannel detector systems, and laser sources with operating wavelength in the ultraviolet, visible or near-infrared region are employed. In FT Raman spectroscopy, the excitation is provided exclusively by near-infrared lasers (1064 nm or 780 nm).

The reason for this 'rivalry' is that the spontaneous Raman scattering is a weak effect and thus it is essential to optimise the experimental set-up. The crucial factor is that the efficiency of the Raman scattering process has one of the highest power dependencies on frequency of any optical effect. This efficiency is proportional to frequency to the fourth power and the intensity of a *Stokes Raman* band of a shift frequency ν_M is governed by

$$I_{RA} \propto (\nu_L - \nu_M)^4 \tag{5.27}$$

Thus, a high frequency excitation source enhances the number of scattered Raman photons dramatically. As an example, let us consider the relative intensity of a 1000 cm^{-1} Raman line excited at 1064 nm and also excited at 514 nm. The first wavelength is available from an Nd:YAG (yttrium aluminum garnet doped with neodymium) diode laser, and the second from an argon ion laser. Using the excitation at 1064 nm the 1000 cm^{-1} Raman band occurs at 1191 nm, and for the excitation at 514 nm the Raman line would occur at 542 nm. Therefore, the enhancement of the 514 nm excitation relative to the 1064 nm excitation would be by a factor $(1191/542)^4 = 23.3$. This means that there are 23.3 times as many photons to detect with the shorter wavelength excitation simply as a consequence of the nature of the scattering process. This can be a very important effect.

Considering this factor, it is obvious that a short wavelength laser source would be the optimum excitation system. Sometimes this statement is true, but often such a source gives unusable data. The reason for this is that many samples, in particular polymers and biological systems, are fluorescent. Even a small bit of fluorescence can mask the much weaker Raman signals completely. As a rule of thumb, many samples are significantly fluorescent with excitation in the 400–550 nm range. At 633 nm, perhaps 10% of the samples fluoresce. At 785–815 nm of the order of 5% will fluoresce, and at 1064 nm only 1–2% of the samples will still present a fluorescence problem. The explanation of this finding is rather simple. The chances that a short wavelength source will excite fluorescence are much greater than those of a long wavelength source. In other words, the use of a NIR laser greatly reduces the likelihood of fluorescence interference. For illustration load the files RAMAN\CHOCO1064 and CHOCO785, which are Raman spectra of chocolate acquired with laser excitation wavelength of 1064 nm and 785 nm, respectively. The spectrum of CHOCO1064 looks fine, but CHOCO785 appears as a broad hump with extremely weak hints of a Raman band.

References

1. Cooley, J. W., Tukey, J. W., Math. Comput., 1965, 19, 297–301.

6 Files

Listed in the *File* menu shown in Fig. 6.1 you will find all commands necessary to manage your files. Besides the basic file operations like loading, unloading, and printing you can also search OPUS files stored on the hard disk. Here, you will also find the *Exit* command to close OPUS.

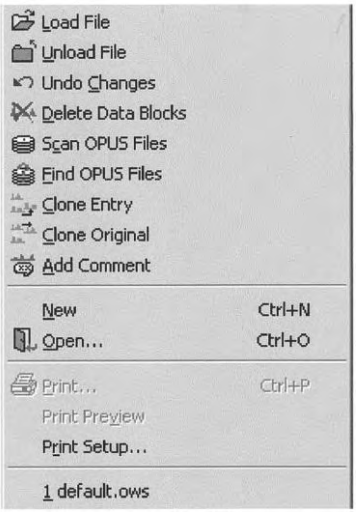

Figure 6.1. The *File* pull-down menu.

6.1 Loading a File

The command *Load File* has already been described in Section 3.1.

6.2 Unloading a File

The dialog box "*Unload Files*" depicted in Fig. 6.2 consists only of a file selection, where the files that should be unloaded have to be entered.

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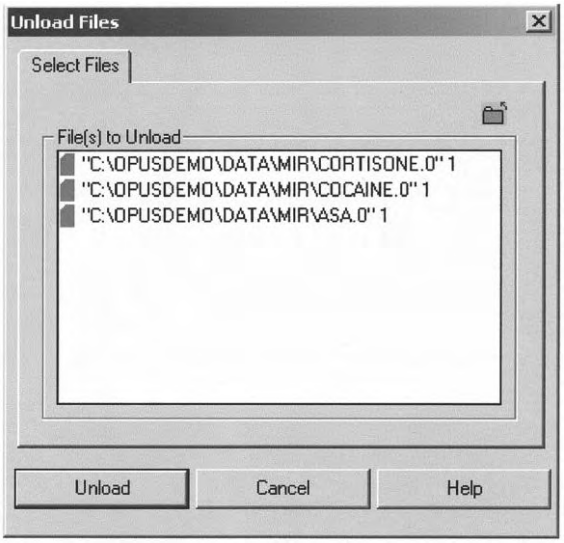

Figure 6.2.
The *Unload* dialog box.

Left-clicking on *Unload* removes the files from the OPUS Browser and the spectrum window. However, upon unloading, the files are not deleted from your hard drive.

6.3 Undo Changes

The dialog box *Undo all changes* is again a file selection box. Clicking on *Restore* revokes all changes made in a data file since it was last saved; the original data will be restored.

6.4 Deleting Data Blocks

As you have seen before, OPUS files usually consist of several data blocks. These can be deleted individually from the file, not only removed from the display!, using the *Delete Data Block* command. All corresponding data of a deleted data block, like search tables and integration results, will also be lost!

6.5 Scan and Find OPUS Files

OPUS offers a powerful tool, the *Find OPUS Files* command, in order to perform search runs on the spectra stored on your hard disk. *Find OPUS Files* searches a database for text strings defined by the user. Before you can use the *Find OPUS Files* command for the first time, you have to build this database. This is done with the *Scan OPUS Files* command

On the first page of the accompanying dialog box (see Fig. 6.3) select the drives you would like to scan. Click on the *Scan Files* button to start the scan. Depending on the amount of spectra building the database and the size of the drives this will take several minutes. While this process is active, "Scan OPUS Files" will be displayed in the Status Bar.

On the second page of the dialog box, as shown in Fig. 6.4, you can limit the information stored in the database by indicating the OPUS parameters you want to include or exclude. Excluding OPUS parameters speeds up the database generation. The abbreviations of the parameters have the following meaning:

CNM	Operator Name
CPR	Copyright Message
EXP	Measurement Experiment
INS	Instrument Type

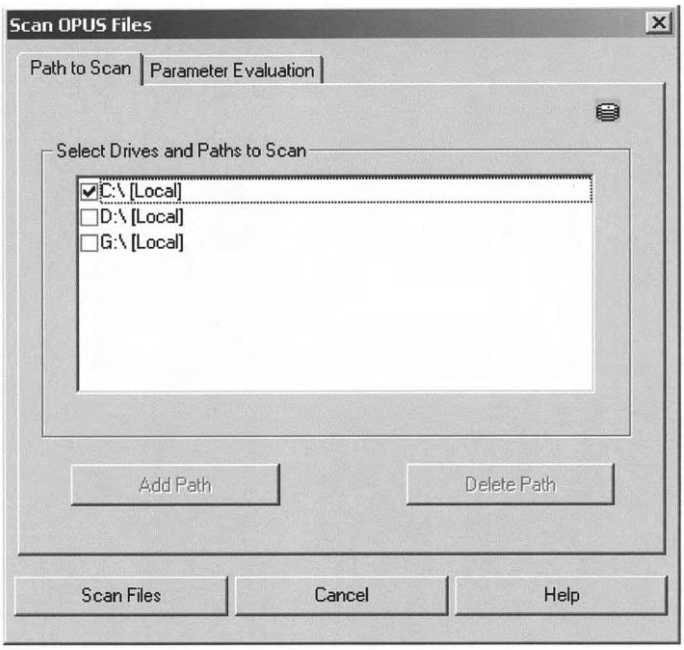

Figure 6.3.
The *Scan OPUS Files* dialog box: *Path to Scan* page.

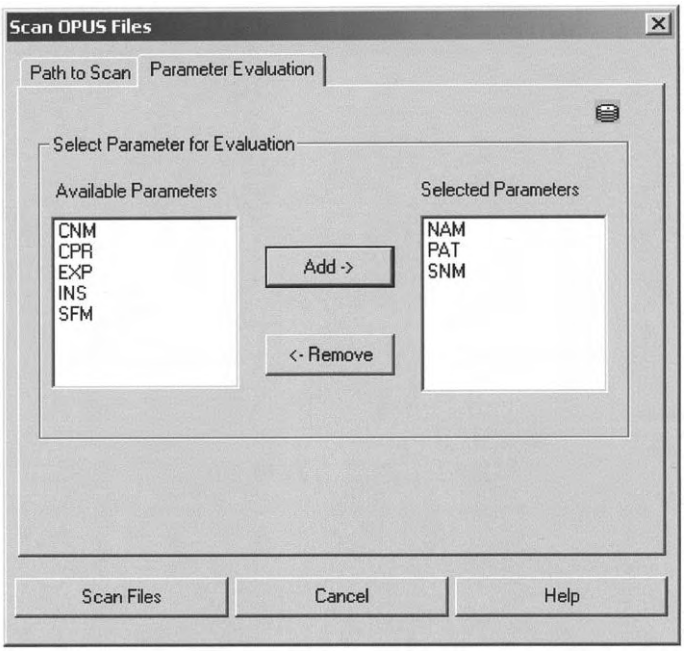

Figure 6.4.
The *Scan OPUS Files* dialog box: *Parameter Evaluation* page.

NAM	File Name
PAT	Path of File
SFM	Sample Form
SNM	Sample Name

As soon as a database has been generated you can search for file names, text strings in files or file parameters. An example is illustrated in Fig. 6.5. If you have added spectra to your hard drive since you generated the database, it is recommended that you update it by clicking *Update Database now* prior to a search run. The time of the last update is displayed to the left of this button.

Enter the string to be searched in the *Find Text in Fields* line. You can specify whether to find exact matches only, any of the words, or all of the words you entered. You can further restrict the search by specifying a date in the *Find in Period* frame. Select between *after, before* or *between* these dates to narrow down your search. Start the query by clicking on *Find in Database*.

All matching spectra are displayed in a list. When the cursor points to a spectrum, the selected parameters and information from the info block are displayed in the table on the right. Select files from the list and load them by clicking on *Load Selected Files*.

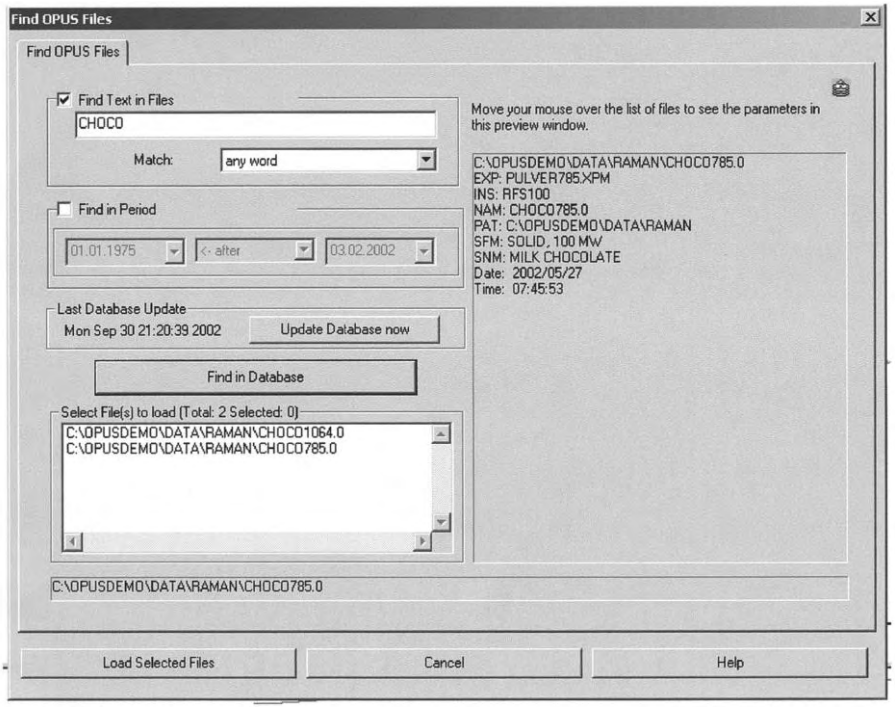

Figure 6.5. The *Find OPUS Files* dialog box.

6.6 Clone Entry and Clone Original

Clone Entry and

Clone Original are used to duplicate spectrum files. Using the command *Clone Entry* you can copy a data file that has been manipulated, as for example a background corrected spectrum. Applying *Clone Original* creates a copy of the original data of such a file. Figure out for yourself how these commands work.

6.7 Add Comment

This command opens a dialog box where you can add a comment to the Audit Trail of a spectrum. An illustration is given in Fig. 6.6. This comment will appear in the *Report Display* under the data block *History.*

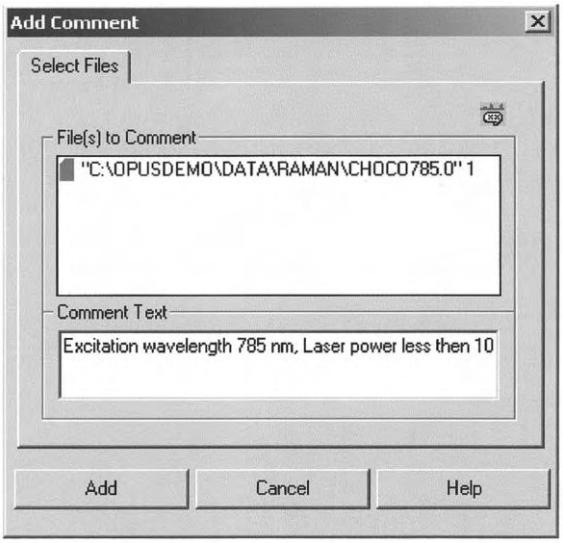

Figure 6.6.
The *Add Comment* dialog box.

6.8 Open

Open (Ctrl+O) opens an existing OPUS workspace and serves to log on to OPUS without exiting the program.

6.9 Printing

Using the *Print* command you can print a report on your default printer. It is only active if a report is open.

6.10 Print Preview

This command is only available for printing reports; it will give you a preview of the printout.

6.11 Print Setup

A default printer can be defined using the dialog box *Print Setup* shown in Fig. 6.7. Here you can specify the paper size and orientation; they will be kept as default for further OPUS printouts.

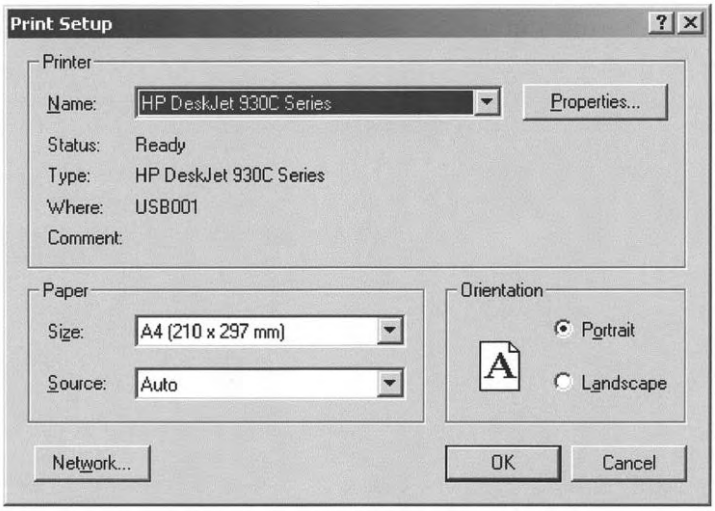

Figure 6.7.
The *Print Setup* dialog box.

7 Edit

The *Edit* pull-down menu lists all features for the information input and functions dealing with chemical structure editing as well as the standard Windows functions like *Copy* and *Paste*. However, only three OPUS commands are active in the demo version (see Fig. 7.1).

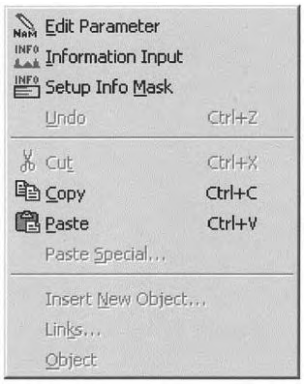

Figure 7.1. The *Edit* pull-down menu.

7.1 Edit Parameter

Essential sample parameters, namely sample name, sample form, operator name, and sample number, which are the input data for running an experiment, can be edited with the *Edit Parameter* command. As usual, load OPUS files into the Browser window and by left-clicking mark either the name or any data block of a file. Further, open the first page of the *Edit Parameter* dialog box, the *Enter Parameter* page, where the sample parameters are listed as shown in Fig. 7.2. In it you can correct misprints, rename parameters, and add information. On the *Axes Labels* page you can set the axes labels and a scaling factor to be used when displaying the spectra.

If the function *Edit Parameter* is not accessible, you should go via the pull-down menu *Setup* → *User Settings* → *21 CFR 11 Rights* to the frame *Validation Option* and deactivate here *Work in Validated Environment* as well as *Work in GLP Mode.*

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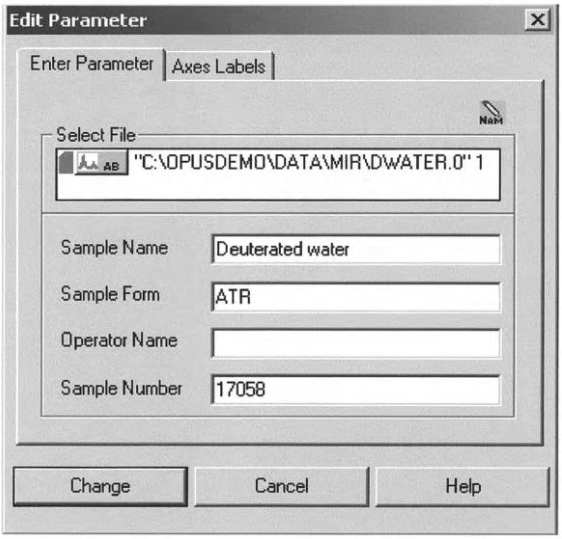

Figure 7.2.
The *Edit Parameter* dialog box: *Enter Parameter* page.

7.2 Information Input

Besides the abovementioned parameters you can save additional information describing your samples by using the *Information Input* function. This input will be saved in an additional information block . You can include this information in a report window as well as in a plot. Note the creation of an information block is mandatory if you want to add the spectrum to a library.

In order to attach information to the data file you first need to create an information mask (refer to Section 7.3). To use an existing information mask for data input, load a spectrum into the Browser and select it. From the *Edit* menu choose *Information Input* and the most recently used information mask will be displayed as shown in Fig. 7.3. Note that you can only choose one spectrum file at a time to enter additional information.

The name of the information mask used for data input is indicated in the *Text Definition* field. If you prefer to use another information mask click on *Load Text Mask*, the relevant dialog box depicted in Fig. 7.4 will appear and from this you can select a different information mask. Enter the information you want to attach to the sample file. You do not need to fill out every field. After inserting the appropriate data use the *Add Information* button to save it. As a result, a new data block will be attached to the OPUS file. Now right-click on the data block icon in the Browser and select *Show Report;* this will open a report window that displays the information you just entered.

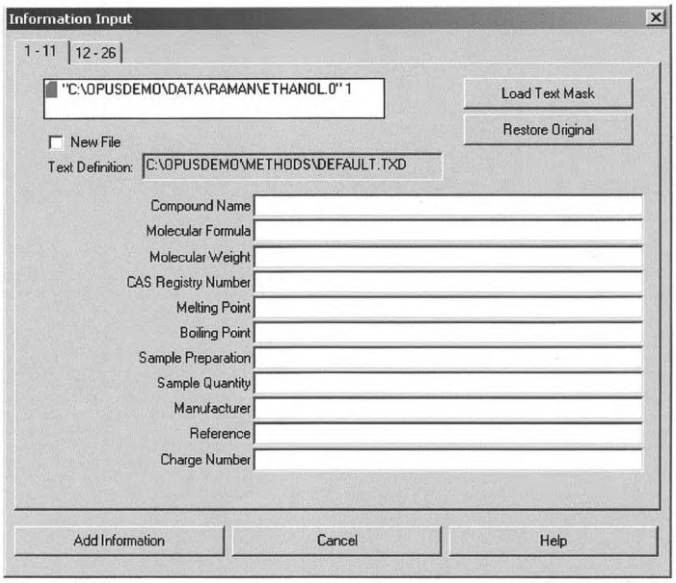

Figure 7.3. The blank *Information Input* mask dialog box.

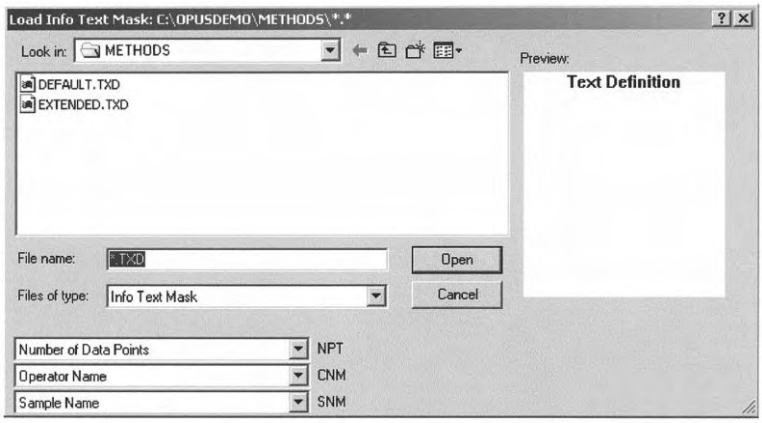

Figure 7.4. The *Load Info Text Mask* dialog box.

Furthermore, you can use the *Information Input* command to edit existing information blocks. If an OPUS file already has an information block attached, it will automatically be opened by the *Information Input* command using the correct information mask. However, if the appropriate mask cannot be found the name of the mask will be highlighted in red. For example, the content of the information block for the MIR spectrum ACETONE is shown in Fig. 7.5. Of course you can alter the information block. Alternatively, you can select a different information mask; in this case all existing information will be discarded.

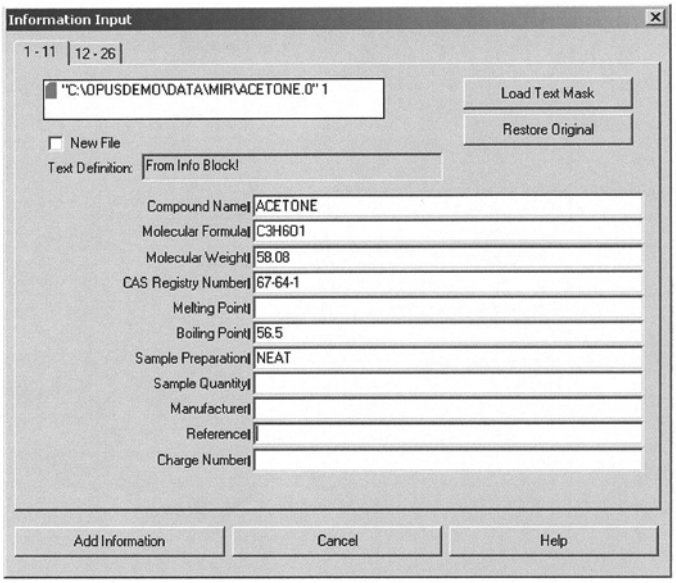

Figure 7.5.
The *Information Input* page for the acetone IR spectrum.

If you accidentally modify or delete fields, you can restore the original data set using the *Restore Information* command.

Instead of attaching an information block to an existing OPUS file you can also create a new file, which then only consists of an information block. In this case check the *New File* box. A new file called INFO*x*.0 will be created, *x* being the number of the file.

7.3 Creating an Information Mask

OPUS allows you to create and modify information masks. An information mask can consist of up to 99 fields. By creating an information mask you define the kind of information you want to save in conjunction with your sample. Thus, you can create various information masks to cover all types of samples. The content and the name of the information mask will be saved together with the information you enter.

Calling up the *Setup Info Mask* function you will see the dialog box shown in Fig. 7.6 where the last active mask is automatically loaded. If no masks exist the dialog box will be empty. You can clear the dialog box using the *Clear All* button. As a result of this, all fields will be colored in red as shown in Fig. 7.7. Now create a new mask by successively entering the names of the fields you want displayed in the mask. Note that blank lines are not allowed. After line 11 you can add another page using the *Next Page* button. Finally,

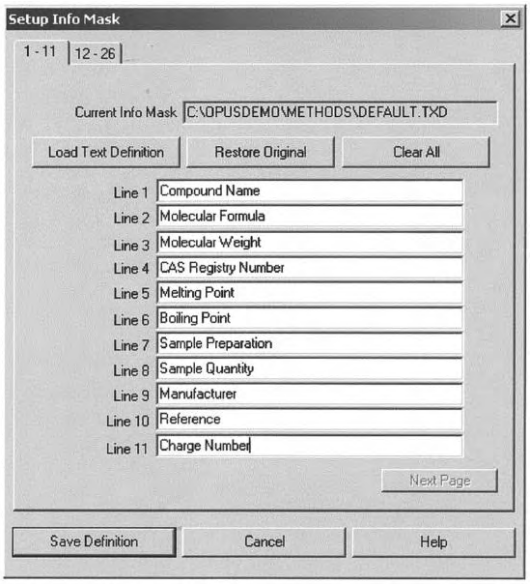

Figure 7.6.
The *Setup Info Mask* dialog box.

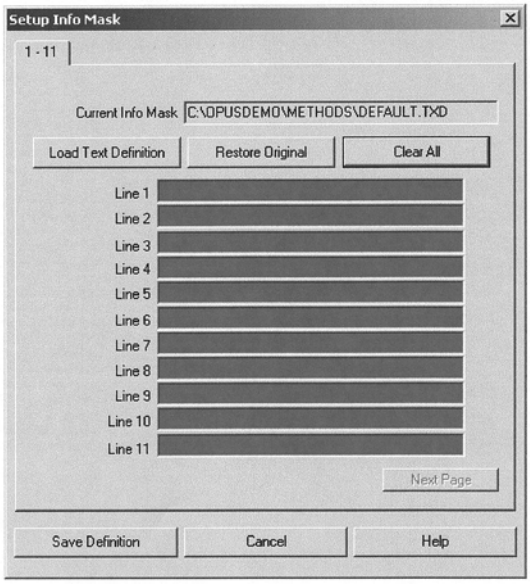

Figure 7.7. The clear *Setup Info Mask* dialog box.

click on *Save Definition* to save the newly generated mask. If a mask with the same name already exists, you will be warned and asked whether you wish to overwrite the existing mask.

To edit an existing mask load it into the *Setup Info Mask* dialog box and modify it. You can discard the changes you have made by clicking *Restore Original*.

7.4 Copy

Using *Copy,* a spectrum or a report can be sent to the clipboard for further processing in terms of another program.

8 View

Via left-click on *View* you have access to a pull-down menu that is used to configure the toolbars, or to toggle the status bar or the browser window on and off (see Fig. 8.1).

Figure 8.1. The *View* pull-down menu.

8.1 Toolbars

In order to configure the toolbars a dialog box (Fig. 8.2) consisting of several check boxes is used. You can access this dialog box by choosing the *Toolbars* command or by right-clicking on the menu or on any toolbar.

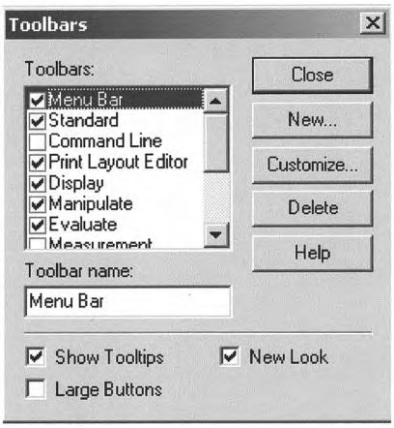

Figure 8.2.
The dialog box for *Toolbars* configuration.

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Marking the check boxes the associated toolbars will be displayed. The toolbars of the workspace DEMO.ows are as follows:

Display

Edit/Info

Evaluate

File

Manipulate

Print

Standard

The icons are here depicted as *Large Buttons* in *New Look*. It is up to you to choose small buttons in new or old look by inactivating the appropriate boxes. The new look version shows no separation between icons in a toolbar. Furthermore, note that it is useful to mark *Show Tooltips*, which means, if you let the cursor rest for a while on an icon, an info text will be displayed, which describes the function of the icon. You can move bars by *Drag and Drop* to any point of the OPUS User Interface. Find out by yourself how the toolbars change by moving to the Spectrum Window or to the Browser Window.

Using the *New* command you can create a new toolbar that meets your personal needs. *Customize* allows you to include additional icons in a toolbar: a second dialog box will open. Switch to the *Commands* page (Fig. 8.4) and select one of the categories. On this page you can select the button you want to add to the toolbar chosen. Drag the icon that you want to include on the toolbar;

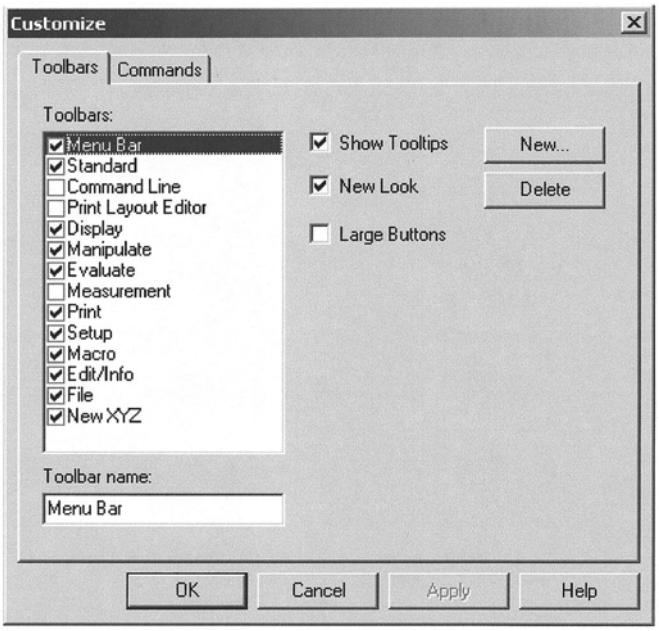

Figure 8.3.
The *Customize* dialog box: *Toolbars* page with the newly created toolbar NEW XYZ.

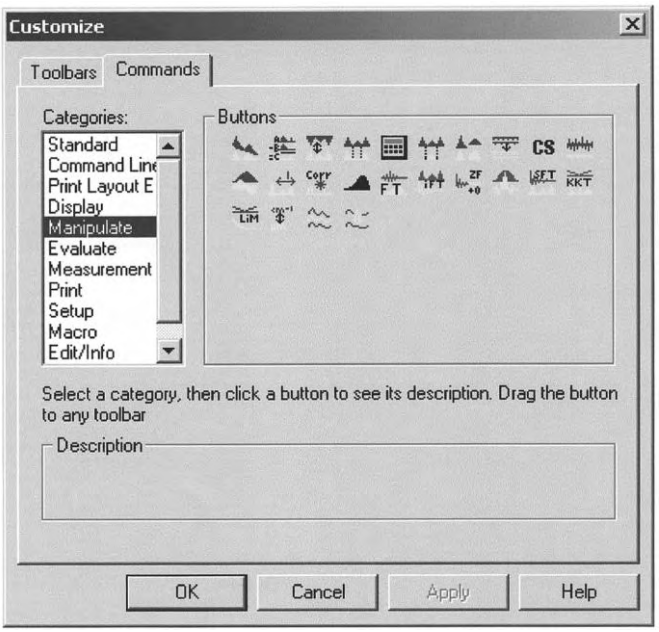

Figure 8.4.
The *Customize* dialog box: *Commands* page.

by dragging an icon of the toolbar to an empty space you can remove this icon from the bar. Of course, you can also delete a toolbar.

In summary, it can be useful to modify the toolbars, but remember that in the frame of OPUSDEMO you cannot save toolbars new configured.

8.2 Status Bar

The status bar on the bottom of the OPUS user interface displays information about programs running in the OPUS environment and their current status. You can activate or deactivate the status bar by checking the *Status Bar* command.

8.3 Browser

By default the OPUS user interface displays the Browser window on the left side. If you deselect the *Browser* command, only the spectrum window and the preview will be shown. Besides these options you can also reposition the browser window (see Section 9.3).

9 Window

The *Window* pull-down menu (Fig. 9.1) allows you to customize the position and appearance of the spectrum and report windows.

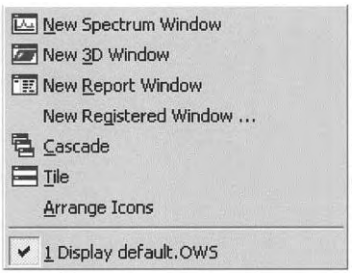

Figure 9.1. The *Window* pop-up menu.

9.1 New Spectrum Window

OPUS not only allows you to display several spectra in the same window, but also to open several windows at the same time, each one capable of holding one or more spectra. The respective command *New Spectrum Window* opens an additional spectrum window indicated by the tab Display defa... at the bottom of the spectrum window. By clicking on the respective tab you can toggle between the windows.

9.2 New Report Window

Blank report windows can be opened using this command. OPUS uses report windows to display information other than spectra or interferograms. For example, the results of a peak pick operation PEAKS and the parameters used can be listed numerically by dragging the peak pick data block to the report window.

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9.3 Cascade and Tile Windows

and

You can choose between cascade or tile arrangement of the different windows. Examples are given in Fig. 9.2 and 9.3.

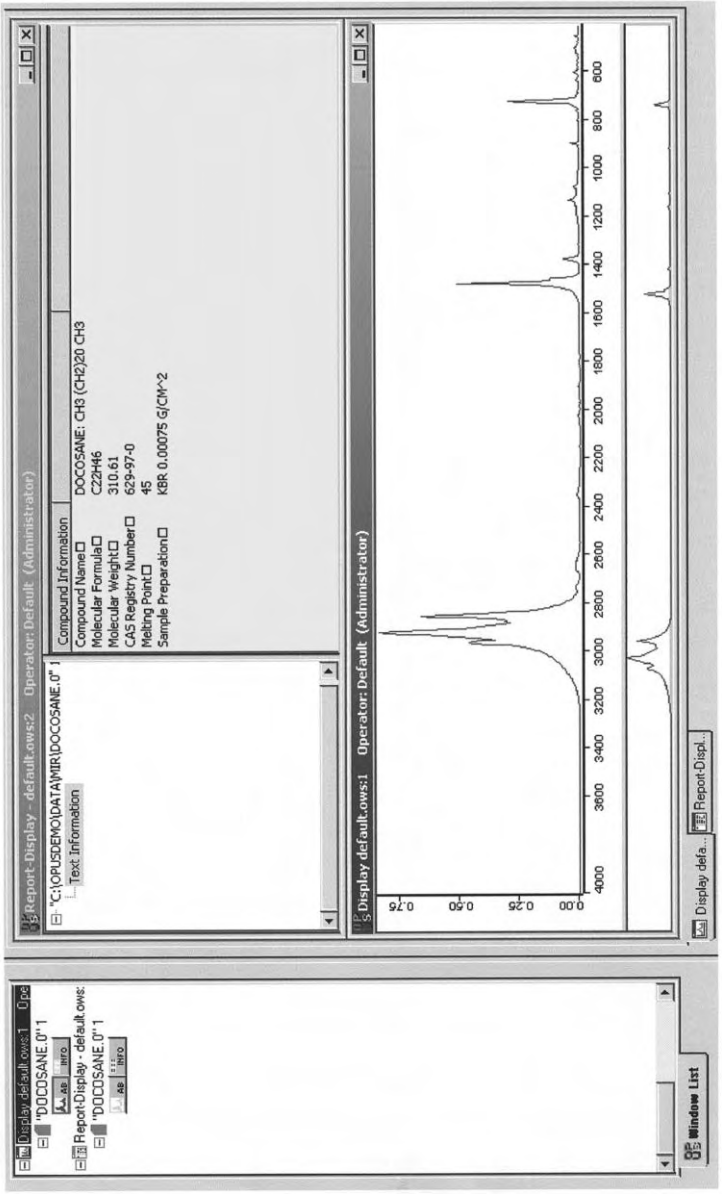

Figure 9.2. The *Tiled* window of the OPUS user interface.

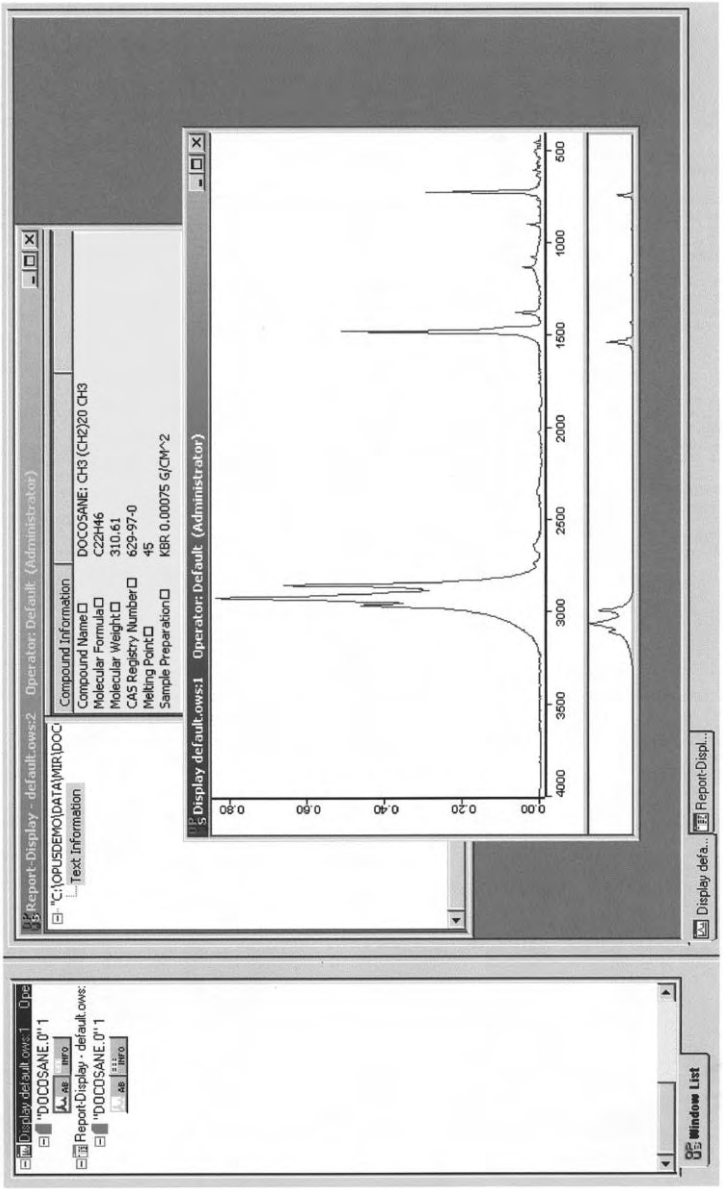

Figure 9.3. The *Cascaded* window of the OPUS user interface.

Figure 9.4. The *Browser* pop-up menu.

Additionally, in combination with the Browser pop-up window (Fig. 9.4), open it by right-clicking on the Browser window, it is possible to customize the OPUS interface. There are three commands, namely:

- *Allow Docking* tags the Browser window to the spectrum window; otherwise the Browser will be displayed in its own window.
- *Hide* is similar to deselecting the *Browser* command in the *View* pull-down menu.
- *Float in Main Window* opens the Browser in a window similar to a spectrum window (maximized and with a tab at the bottom).

10 Manipulating

This chapter deals with the discussion of all OPUS data processing and manipulation procedures, which are shown in Fig. 10.1. These functions allow you to transform or analyze the spectral data after it has been acquired. The results of all operations are temporary files, i.e. existing only in the computers memory, called work files that must be saved explicitly. If you exit OPUS without saving the work files to disk, the results of the data manipulation will be lost. OPUS warns you in case you attempt to shut down the program.

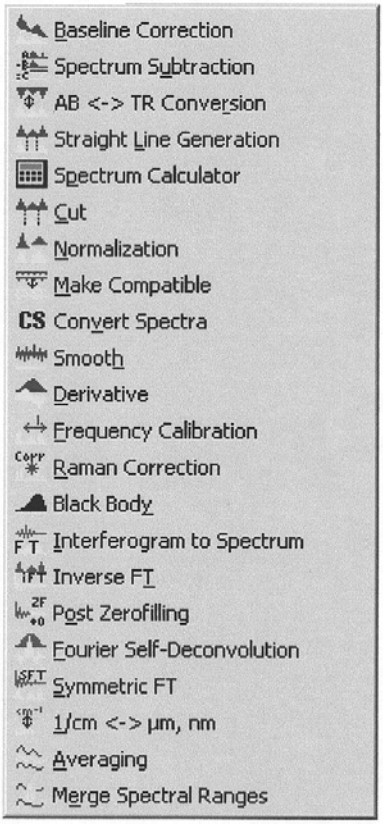

Figure 10.1. The *Manipulate* pop-up menu.

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10.1 Baseline Correction

This function performs a baseline correction on the selected spectra. First load the spectrum that you would like to correct on the first page of the *Baseline Correction* dialog box shown in Fig. 10.2. The baseline correction can be performed simultaneously for several spectra.

You can either set the baseline points manually by switching to the interactive mode *(Start interactive mode* button) or have them set by the program. In the first case a dual window opens, containing the original spectrum on the top of the panel and the corrected spectrum at the bottom. You can choose between fitting a straight line or a parabola to the data. An example is given in Fig. 10.3 for the IR spectrum of the compound B. To add a baseline point, double-click on a selected point of the original spectrum in the top window. The result of the correction will be immediately displayed in the second window. If you want to remove a baseline point, double-click on it a second time. Use the *Zoom* function (see Section 3.3) in combination with the interactive mode to enlarge parts of the spectrum; an example is given in Fig. 10.4. You can save the result of the correction by clicking on the *Store* button.

In order to automatically correct the baseline of the spectrum, switch to the *Select Method* page (Fig. 10.5) after loading the spectra. Here, you have to specify the correction method and the number of baseline points to be used. As an option you can exclude the spectral region that contain CO_2 bands. In this case, data points between 2400 and 2275 cm^{-1} and between 680 and 660 cm^{-1} are not taken into account in the calculation. This feature is available for the *Automatic* option only.

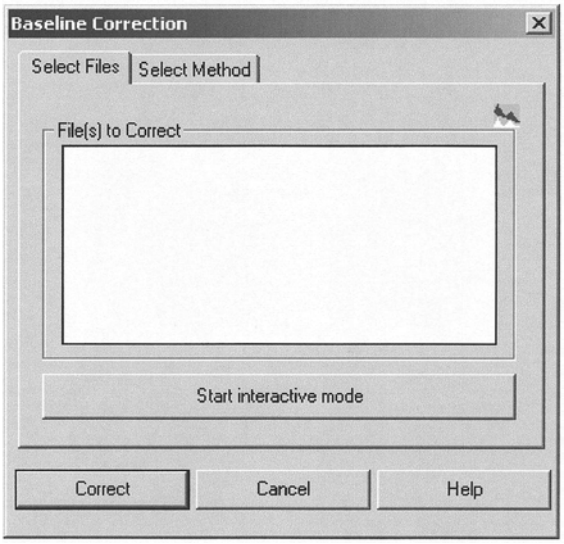


Figure 10.2. The *Baseline Correction* dialog box: *Select Files* page.

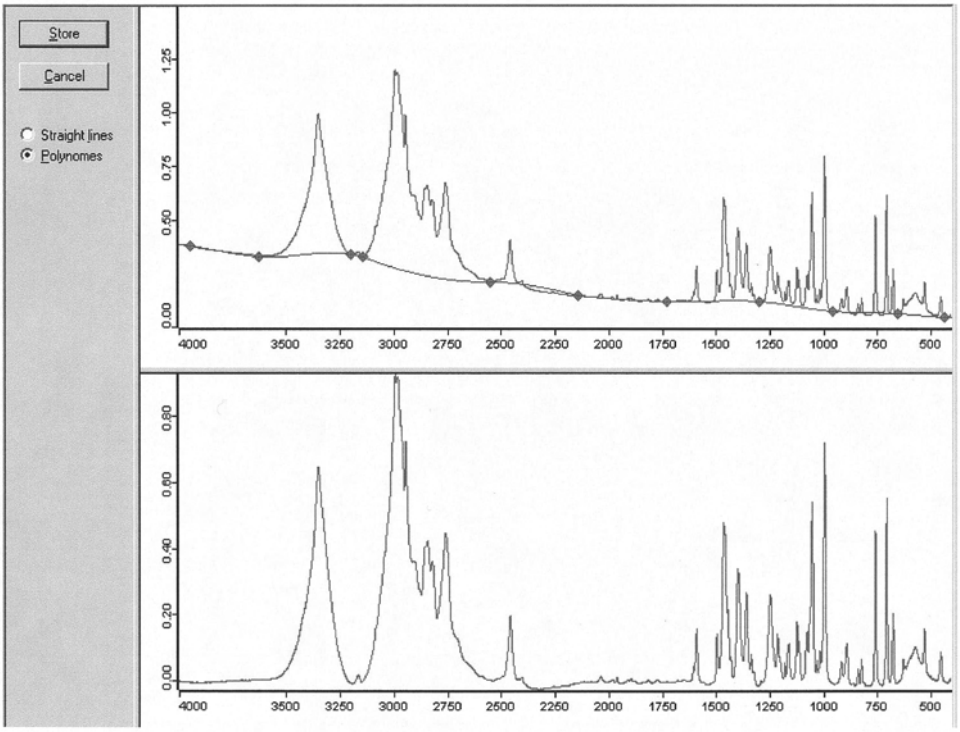


Figure 10.3. The *Baseline Correction* interactive mode: The original IR spectrum of the compound B is displayed in the top window; the baseline corrected one in the bottom window.

The number of baseline points can be varied by clicking on the *Number of Baseline Points* input field and entering a value between 10 and 200 (unless the function range selected was too small, a minimum of 10 and a maximum of 200 baseline points will be used). The default value is 64. Choose a correction method and start the computation by clicking on *Correct*.

For construction of the baseline, the spectrum is divided into n ranges (n being the number of baseline points) of equal size. In the case of absorbance spectra the minimum y-value of each range is determined. Connecting the minima with straight lines creates the baseline. Starting from "below", a rubber band is stretched over this curve. The rubber band is the baseline. The baseline points that do not lie on the rubber band are discarded.

If you choose *Scattering Correction* the baseline is constructed in such a way that at each point the slope of the baseline must be negative for an absorbance-like spectrum (provided that the spectrum is displayed in decreasing wavenumbers from left to right).

As examples, Fig.10.6 shows the original spectrum of the compound B, here in the spectral range between 4000 and 1800 cm^{-1}, and the spectrum after baseline

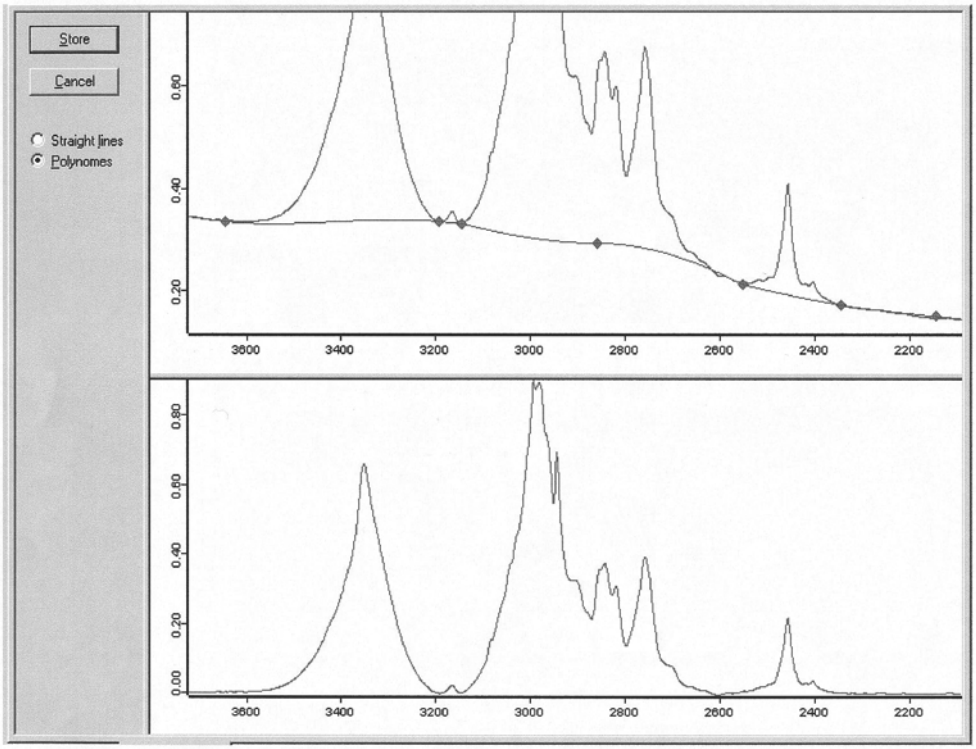


Figure 10.4. The *Baseline Correction* interactive mode after using the *Zoom* function.

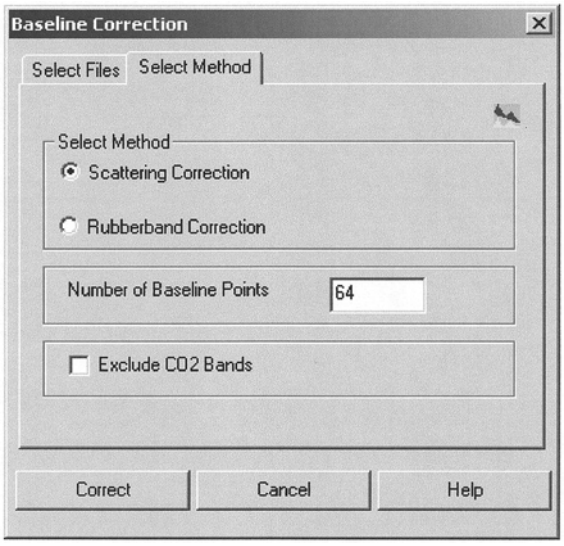


Figure 10.5. The *Baseline Correction* dialog box: *Select Method* page.

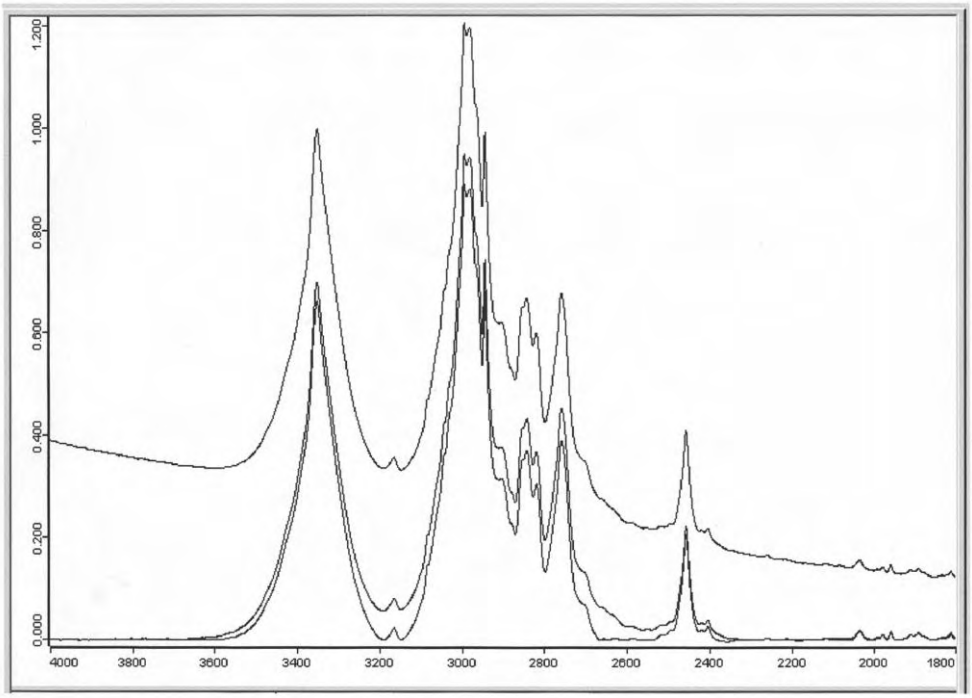


Figure 10.6. The IR spectrum of compound B in the spectral range between 4000 and 1800 cm^{-1} prior to and after *Baseline Correction*. From top to bottom original spectrum, "scattering" corrected spectrum, and "rubber band" corrected spectrum.

correction using the scattering and rubber band method with 64 baseline points, respectively. By subtracting the corrected spectrum from the original spectrum, the baseline can be obtained (see Fig.10.7).

Baseline corrections can also be performed on transmittance spectra.

10.2 Spectrum Subtraction

Click this icon to open the *Spectrum Subtraction* dialog box (Fig. 10.8). Fractions of one or more spectra can be subtracted from another spectrum. A linear offset can also be removed; for example

Resulting spectrum (v) = Multiple component spectrum (v)
– (Factor1) times (single component spectrum (v))
– (Factor2) times (single component spectrum (v))
– Offset (10.1)

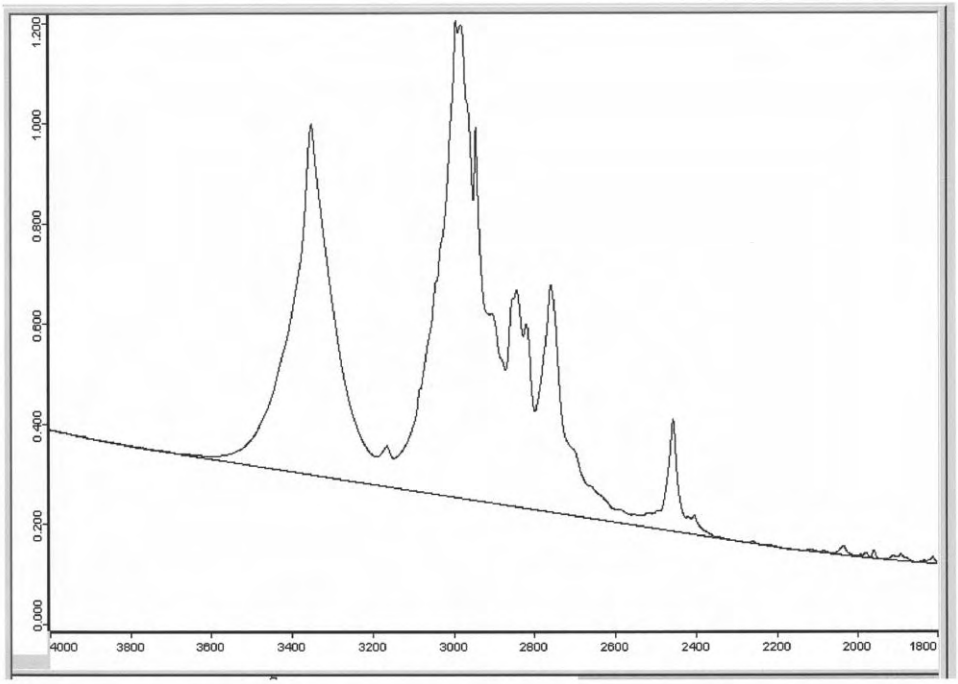


Figure 10.7. The original IR spectrum of the compound B and the "rubber band" baseline.

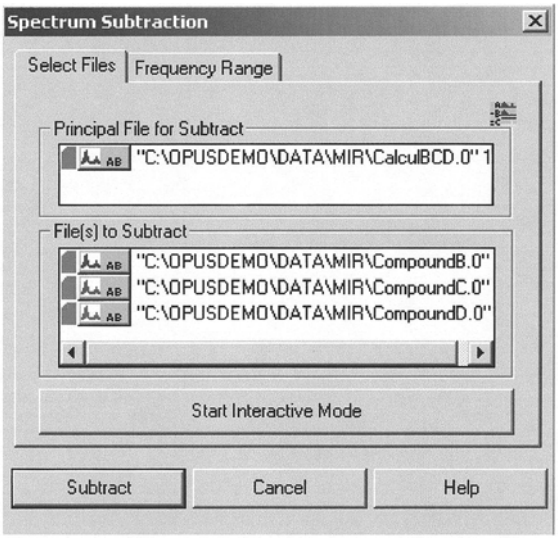


Figure 10.8. The *Spectrum Subtraction* dialog box.

Notice that these kinds of calculations are only appropriate for absorbance spectra and Raman spectra. Transmittance spectra are converted automatically to absorbance spectra. If the original spectrum was a transmittance spectrum, the resulting spectrum is converted to transmittance after the subtraction.

There are two modes of operation, namely an automatic one started by *Subtract* and an interactive one launched by *Start Interactive Mode.* In the automatic mode, the difference spectrum with automatically determined multiplication factors is calculated from a least squares analysis over the displayed wavenumber range. The range of wavenumbers used is the largest range present in all spectra. The interactive mode allows you to set the multiplication factors manually.

Let us demonstrate how these procedures work. Load the MIR spectra CalculBCD, CompoundB, CompoundC, and CompoundD. The spectrum CalculBCD is generated as a superposition of the spectra of the compounds B, C, and D as follows

$$\text{CalculBCD} = 2.5\ \text{CompoundB} + 1.3\ \text{CompoundC} + 0.7\ \text{CompoundD} \tag{10.2}$$

This spectrum can be easily calculated by using the *Spectrum Calculator* (see Section 10.5). It should represent a generic sample consisting of an unknown amount of the three components.

Now, we try to analyze this spectrum using spectral subtraction. In order to avoid lost spectra, we clone the original ones and handle the clones. Open the *Spectrum Subtraction* dialog box (Fig. 10.8) by clicking on the appropriate icon. Because the automatic mode tries to minimize the least squares deviation in the difference spectrum, the optimum result can only be achieved if all single components are subtracted from a mixture at the same time, and consequently we expect the subtraction result to be equal to zero. The spectrum of the 'mixture' will be entered in the top box and the spectra of the three components in the bottom box. Then, specify the spectral region in which the subtraction should be performed on the second page of the dialog box. Click *Subtract*, the automatic subtraction will be performed and the subtraction data block SUBTR. will be stored with the *Principal File.* This block contains the factors used to subtract the spectra and the resulting spectrum. A report window listing these parameters can be opened by right-clicking on the subtraction data block (see Fig.10.9). The mark in the *Modified* column indicates which file has been altered.

The dialog box of the interactive mode (Fig. 10.10) consists of three small display boxes on the left panel in which you enter the spectrum of the 'mixture', the spectrum of the component B, and the multiplication factor (here 2.3), respectively. To scale the spectra be sure to place a mark in the *Scale Spectra with factor* box. On the right panel, the two spectra will be displayed in the top window and the result of the subtraction is shown in the bottom window. As in a regular spectrum window, you can use the zoom function to magnify a specific region of the spectra in both windows. You can set the

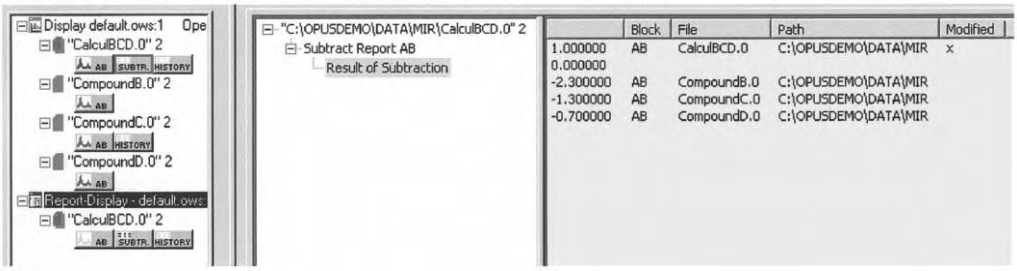


Figure 10.9. The report of a spectra subtraction.

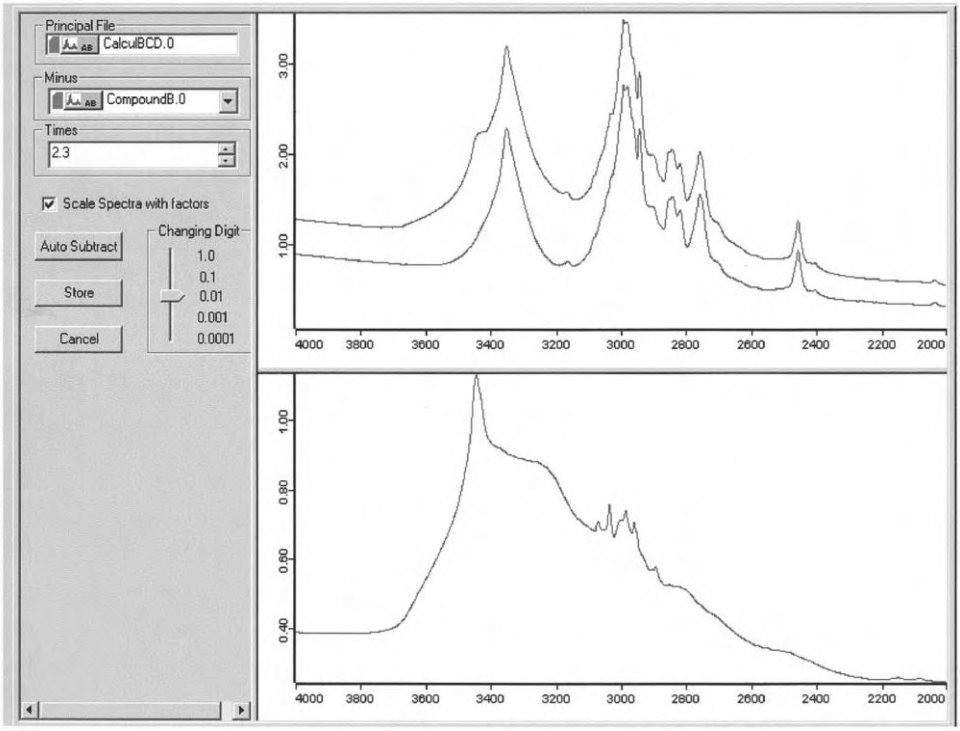


Figure 10.10. The dialog box of the interactive mode for spectrum subtraction.

number of decimal places of the factors with the *Changing Digit* switch. If you prefer to switch to automatic subtraction, use the *Auto Subtract* button. Make sure to save your resulting spectrum in the *Principle File* by clicking on *Store* before you exit the dialog box.

10.3 Conversion of IR Spectra

This function converts an absorbance spectrum to a transmittance spectrum and vice versa. In the dialog box (Fig. 10.11) the type of conversion can either be stated explicitly or the conversion can be done automatically.

If you explicitly choose a specific direction, the function is applied only to those selected files that do not have the required output data block. Thus, this function makes it possible to set a uniform spectrum block for many data files. Start the procedure by clicking on *Convert.*

The equation used for the conversion is:

$$AB = -\log_{10}(TR) \tag{10.3}$$

Transmittance values below 10^{-5} are set to 5 absorbance units.

For the reverse conversion OPUS uses:

$$TR = 10^{-AB} \tag{10.4}$$

As an example, a converted absorbance spectrum together with the input spectrum are illustrated in Fig. 10.12.

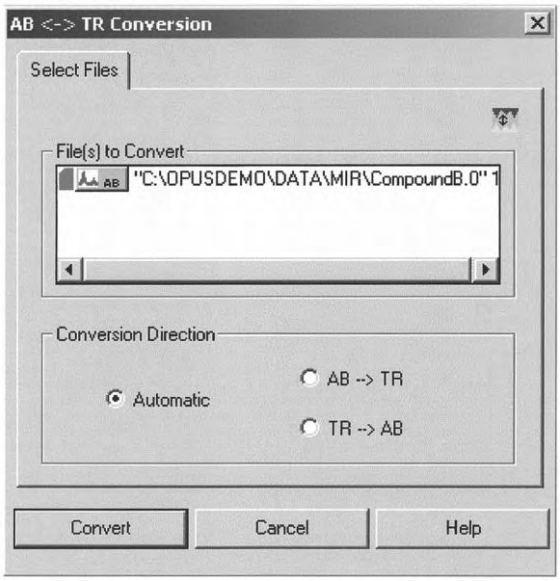


Figure 10.11. The dialog box of the *AB ↔ TR Conversion* for IR spectra.

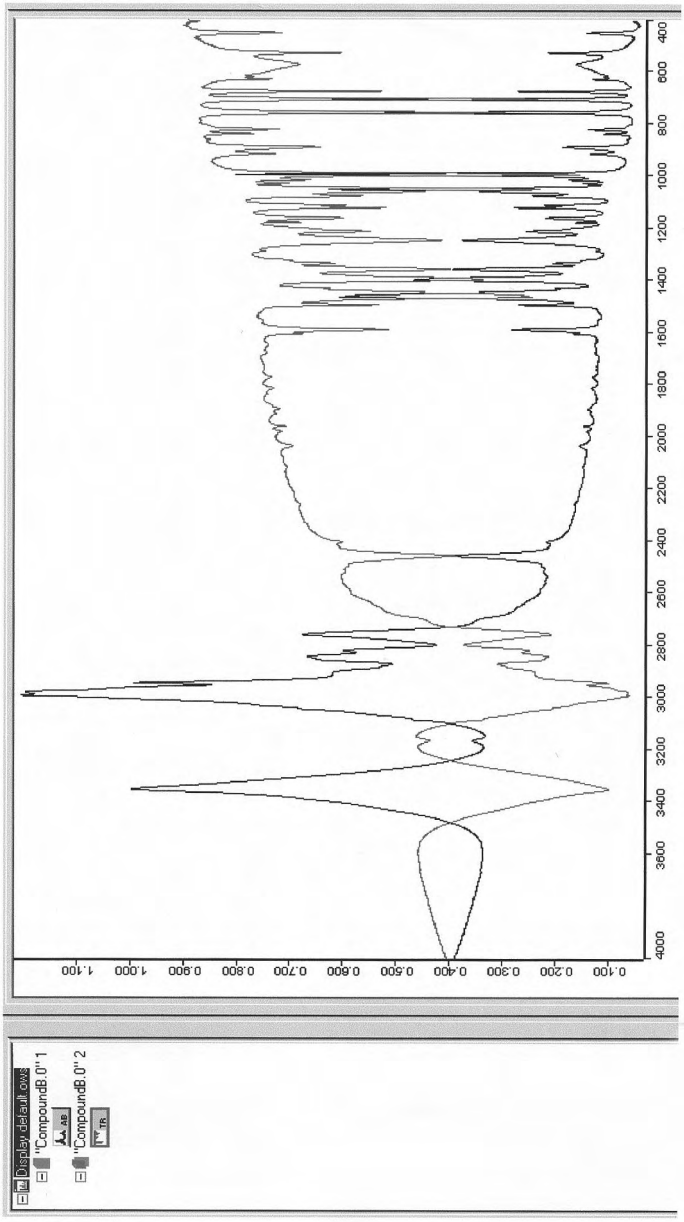


Figure 10.12. Converted absorbance spectrum together with the input transmittance spectrum.

Figure 10.13. The *Straight Line Generation* dialog box.

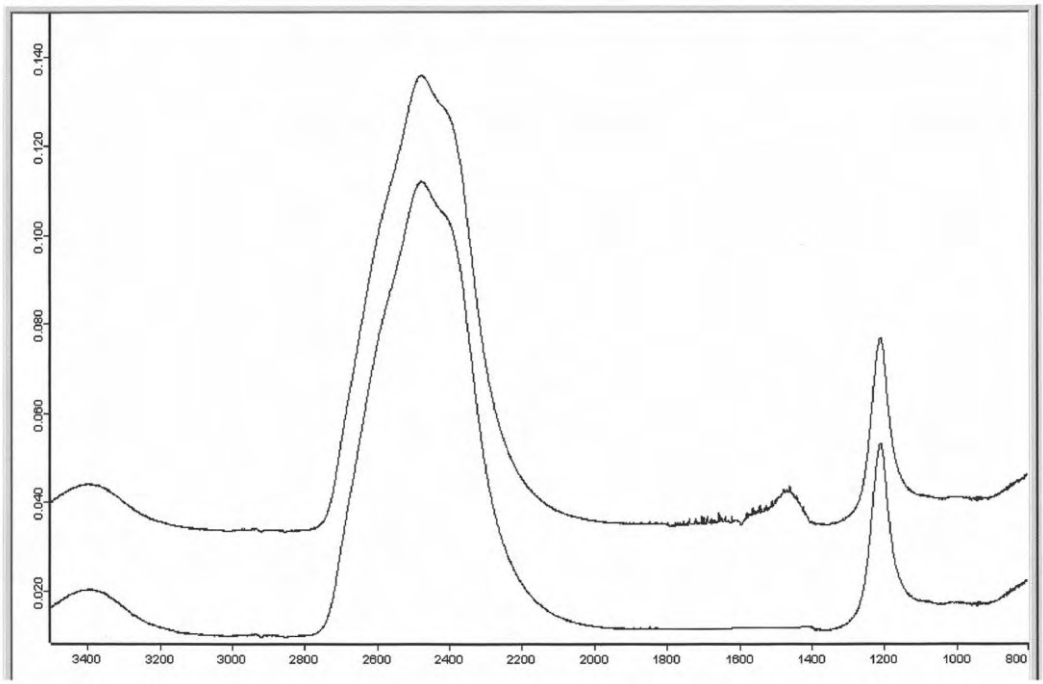


Figure 10.14. The IR spectrum of D_2O: top, original spectrum; bottom, spectrum with straight line inserted in the spectral region between 1800 and 1400 cm^{-1}.

10.4 Straight Line Generation

This function can be used to correct artifacts in a spectrum or to make corrections in the interferogram to reduce fringe effects. Drag the spectrum onto the window of the respective dialog box (Fig. 10.13) and enter the spectral range in which you want a straight line to be generated. Start the function by clicking on *Generate.*

In the example given in Fig. 10.14 a straight line is inserted in the spectral region between 1800 and 1400 cm^{-1}.

10.5 Spectrum Calculator

As the heading implies, the calculator is mainly designed to mathematically manipulate spectra, however numerical calculations can also be performed. As you can see in Fig. 10.15, the shape looks like a pocket calculator.

The function block on the top (Fig. 10.16) comprises the trigonometric functions sine, cosine, and tangent. Use the *Backspace* ← button to delete your last input. Click on *Pi* to enter the number π.

The *Shift* and *Hyp* button make the inverse circular functions (Fig. 10.17) and the hyperbolic functions (Fig. 10.18), respectively, available.

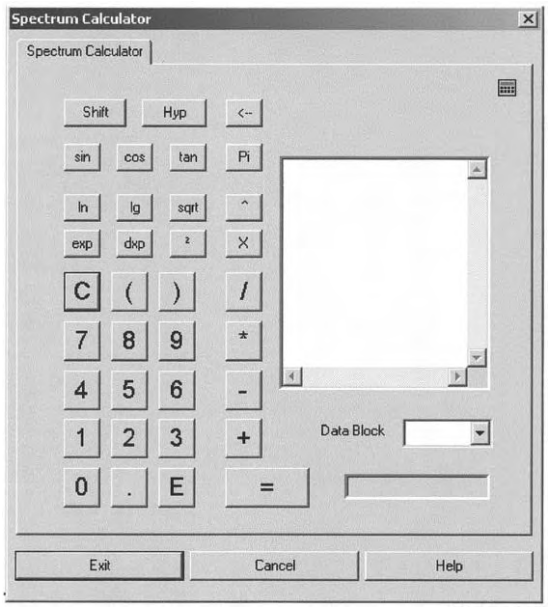


Figure 10.15.
The *Spectrum Calculator.*

Figure 10.16. The function block on the top of the calculator.

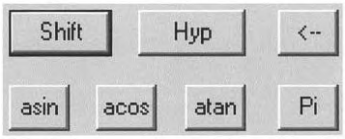


Figure 10.17. The block of the inverse circular functions.

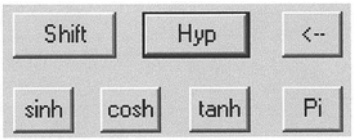


Figure 10.18. The block of the hyperbolic functions.

Figure 10.19. The block of other mathematical functions.

Additional mathematical functions (Fig. 10.19) are as follows:
ln is the natural logarithm,
lg is the logarithm to the base 10,
sqrt is the square root function $\sqrt{\ }$,
exp is the exponential function,
dxp takes the following entry to the power of x (i.e. 10^x),
and "^{2}" and "^" are power functions like $3^2 = 9$ and $3\text{^}3 = 3^3 = 27$.

C clears the displays. The other buttons have their usual meaning.

You can enter data either by using the numerical keypad of your computer or by clicking the keys of the spectrum calculator with the mouse. The button = starts the calculation. The result will be displayed in the grayish window on the bottom right.

You can drag spectra from the browser to the selection window. The spectra can be processed directly by using the mathematical functions. To illustrate the use of the spectrum calculator, Fig. 10.20 shows the input feature for calculating the spectrum CalculBCD according to Eq. (10.2).

If the result of your computation is a spectrum, it will overwrite the original spectrum file used for the calculation. Furthermore, it will be displayed in the spectrum window, if it fits the displayed region.

If a calculation is performed with different spectrum types, the resulting spectrum generally receives a copy of the data block of the first spectrum in the formula.

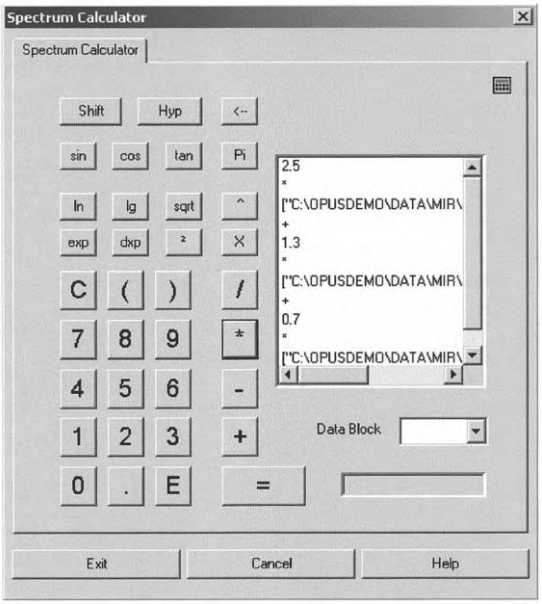


Figure 10.20. The input data for calculating the spectrum CalculBCD according to the formula 10.2.

Notice the peculiarity of the "*x*" function that represents the wavenumber values of the spectral data points. In some cases it will be necessary to calculate wavenumber-dependent expressions. Using this function you can do this. For example, the expression

$$\sin(x/100) + [\text{INDIGO.0:TR}] - [\text{INDIGO.0:TR}] \tag{10.5}$$

uses the group ([INDIGO.0:TR] – [INDIGO.0:TR]) to generate a spectrum with zero intensity. Only the wavenumber limits and the data point spacing will be used by the "*x*" function**.** The result of the operation (10.5) will be a sine wave.

On the other hand, evaluating the expression

$$\sin(x/100) - [\text{INDIGO.0:TR}] \tag{10.6}$$

would result in a sine wave with the intensity values of the spectrum added.

The spectrum calculator should not so much be thought of as a regular calculator, but rather as a tool for evaluating mathematical expressions.

10.6 Cut

By using *Cut* you can narrow the wavenumber range of a data file. Define the spectrum and spectral range as usual, and then click on the *Cut* button to start the function. Figure 10.21 shows the IR spectrum of pyrrole in the

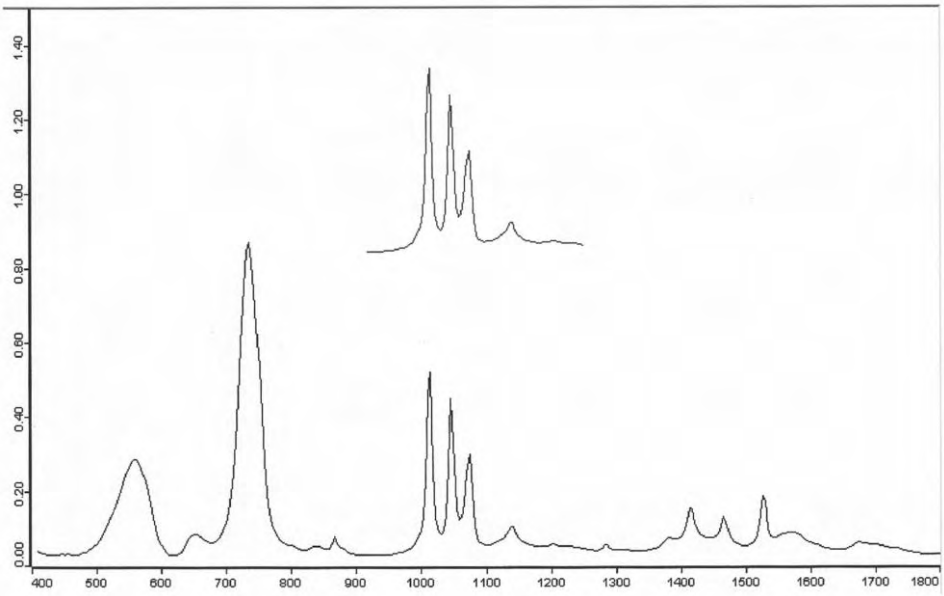


Figure 10.21. The IR spectrum of pyrrole in the spectral range 400–1800 cm^{-1}: bottom, original spectrum; top, a cut-out part of the spectrum.

spectral range 400–1800 cm^{-1} and a region that was obtained from the original spectrum by using the *Cut* function. For illustration the cut-out part is shifted.

10.7 Normalization

This function performs normalizations and offset-corrections of spectra. Three different methods are available for spectrum normalization:

- *Min–Max normalization*

The spectrum is scaled so that the minimum occurring y-value is set to zero and the maximum one is placed at 2 absorbance units. Transmittance type spectra are normalized in a way that the minimum occurring y-value lies at 0.01 and the maximum occurring y-value at 1.

- *Vector normalization*

The average y-value of the spectrum y_m is calculated first:

$$y_{\mathrm{m}} = \frac{\sum_k y_k}{N} \tag{10.7}$$

where N is the number of data points.

This average value is then subtracted from the spectrum, which has the effect of centering the spectrum around $y = 0$:

$$\hat{y}_k = y_k - y_m \tag{10.8}$$

The sum of the squares of all $\hat{y}_k$-values is then calculated and the spectrum is divided by the square root of this sum:

$$Y_k = \frac{\hat{y}_k}{\sqrt{\sum_k (\hat{y}_k)^2}} \tag{10.9}$$

That means that the vector norm of the resulting spectrum is 1:

$$\sum_k (Y_k)^2 = 1 \tag{10.10}$$

- *Offset correction:*

The spectrum is shifted so that the minimum occurring y-value is set to an extinction of zero.

In the relevant dialog box you have to define the spectrum and wavenumber range as usual, and then click on the *Normalize* button to start the function. Figure 10.22 illustrates the *Min-Max normalization* for the IR spectrum of

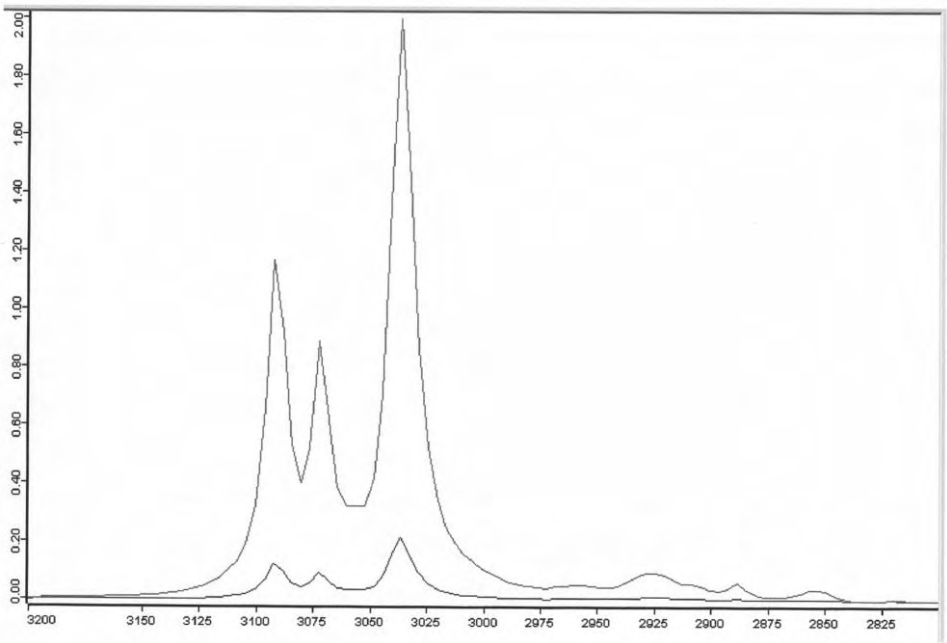


Figure 10.22. The IR spectrum of benzene in the spectral range 3200–2800 cm^{-1} prior to and after *Min–Max Normalization*: bottom, original spectrum.

benzene in the spectral range 3200–2800 cm^{-1}. Find out how the other versions of normalization affect the spectrum.

10.8 Make Compatible

This function changes the data point spacing of the selected files to match that of the "Principal File". The related dialog box is shown in Fig. 10.23. If the file limits of a selected spectrum lie (partially) outside the file limits of the principal file, the selected file is cut accordingly. The files in the file selection bar are manipulated so that the x-values of the spectra are made compatible with those of the principal file. As a result, the wavenumber x-axis points are shifted.

There are two methods of obtaining the y-values corresponding to the new x-values.

- *Interpolation* – The method of quadratic interpolation is applied.
- *Reduce Resolution* – The new y-values are calculated by integration of the original values.

The *Interpolation* method preserves the curve shape better while *Reduce Resolution* keeps the peak positions more constant.

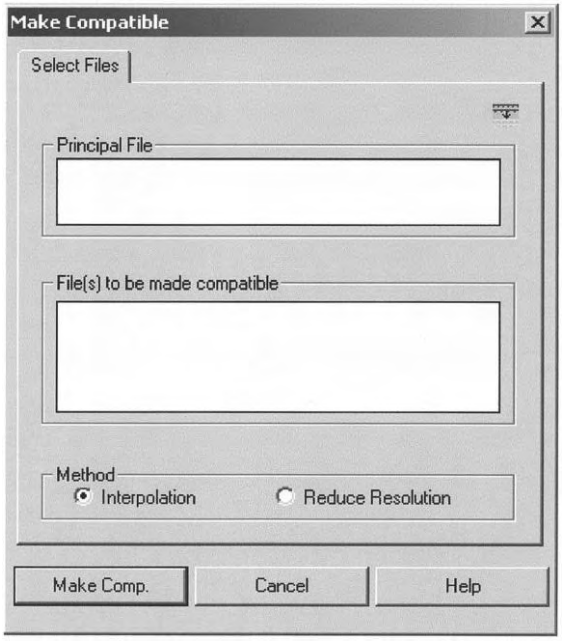


Figure 10.23. The *Make Compatible* dialog box.

Let us consider an example:

The principal file exhibits wavenumber limits 1000 and 5000 cm^{-1}. The number of data points is 4001, i.e. 1 cm^{-1} resolution. For the selected file, the wavenumber of the first point is 4000.5 cm^{-1}, the wavenumber of the last point is 200.5 cm^{-1}, and there are 1901 data points, i.e. 2 cm^{-1} resolution. The *Make Compatible* function creates a new spectrum whose first wavenumber point is at 1000 cm^{-1}, last point at 4000 cm^{-1}, and the number of data points is 3001, i.e. the resolution is 1 cm^{-1}.

The principal file determines the *x*-direction of the new spectrum: high-to-low or low-to-high wavenumber values.

10.9 Spectrum Conversion

CS This function performs several data conversions mainly involving the *y*-axis. When choosing the spectra to convert, certain restrictions, depending on the conversion selected, may apply. If you try to select a spectrum that is not in accordance with the conversion method chosen, a danger sign will be displayed (see Fig. 10.24).

Define the spectrum and wavenumber range as usual, choose a conversion method and click on the *Convert* button to start the function.

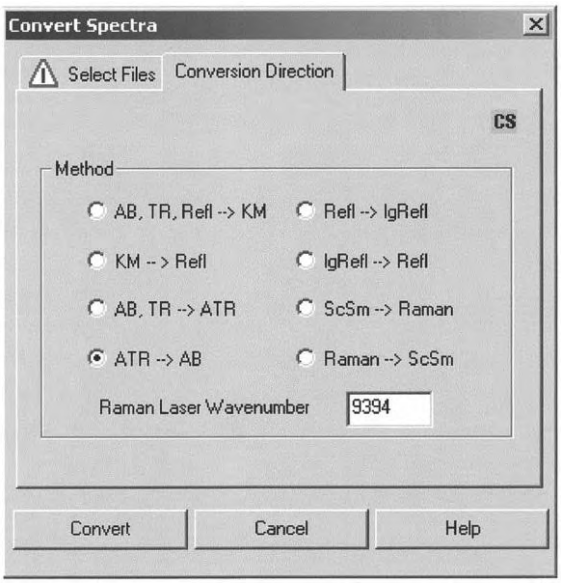


Figure 10.24. The *Convert Spectra* dialog box. Mismatch of method and spectrum because the file selected is an absorbance spectrum.

10.9.1 Conversion to Kubelka–Munk: *AB, TR, REFL* → *KM*

The Kubelka–Munk type is designed for spectra that are measured in diffuse reflectance. The spectral intensities in Kubelka–Munk units are more linear in concentration than those in absorbance units. The conversion formula is

$$KM = \frac{(1 - REFL)^2}{2REFL} \tag{10.11}$$

TR can also be used instead of *REFL*. Absorbance spectra are first converted to transmittance spectra. The smallest value allowed for transmittance is 0.001%. This corresponds to a Kubelka–Munk value of about 500.

10.9.2 Conversion to Reflectance Spectra: *KM* → *REFL*

In this case, the following conversion formula is applied:

$$REFL = 1 + KM - \sqrt{(2KM + KM^2)} \tag{10.12}$$

10.9.3 Conversion to ATR Spectra: *AB, TR* → *ATR*

In attenuated total reflection (ATR) measurements, the depth of penetration, d_p, of the IR beam for a non-absorbing medium is a function of the wavelength λ:

$$d_p = \frac{\lambda/n_1}{2\pi\left[\sin\delta - (n_1/n_2)^2\right]^{1/2}} \tag{10.13}$$

where n_1 is the refraction index of the sample, n_2 is the refraction index of the ATR crystal, and δ is the angle of incidence. In other words, the depth of penetration is inversely proportional to the wavenumber. Therefore, to convert an absorbance or transmittance spectrum to an ATR spectrum the following expression is applied:

$$ATR = AB\frac{x}{1000} \tag{10.14}$$

where x represents the wavenumber. Transmittance spectra are first converted to absorbance spectra.

10.9.4 Conversion to Absorbance Spectra: *ATR* → *AB*

For this conversion the following formula is used

$$AB = ATR\frac{1000}{x} \tag{10.15}$$

10.9.5 Taking the Logarithm of Spectra: *Refl* → lg*Refl*

When taking the logarithm of spectra, reflectance values below 0.00001 will be levelled to a lg(*Refl*) intensity of 5.

10.9.6 Conversion of Spectra in Logarithms: lg*Refl* → *Refl*

The equation used is

$$R = 10^{-\log(R)} \tag{10.16}$$

10.9.7 Conversion of Single Channel Raman Spectra: *ScSm* → *Raman*

OPUS uses *ScSm* to indicate a single channel sample spectrum. The start and end wavenumber of a single channel Raman spectrum will be converted using the equations:

$$FXV(Raman) = RLW - FXV(ScSm) \tag{10.17}$$

$$LXV(Raman) = RLW - LXV(ScSm) \tag{10.18}$$

RLW stands for the wavenumber of the excitation laser; this value will be written to the instrument parameter block. *FXV* and *LXV* represent the start and end wavenumbers, respectively.

10.9.8 Conversion of Raman Spectra: *Raman* → *ScSm*

Here the equations used for the conversion are:

$$FXV(ScSm) = RLW - FXV(Raman) \tag{10.19}$$

$$LXV(ScSm) = RLW - LXV(Raman) \tag{10.20}$$

The *RLW* parameter is taken from the instrument parameter block of the Raman spectrum.

Let us illustrate this conversion. The Raman spectrum of diamond shows a sharp band at 1331 cm^{-1}. Assuming $RLW = 9394$ cm^{-1} this band appears at 8063 cm^{-1} in the single channel spectrum. As another example, Fig. 10.25 shows both types of spectra for ethanol: on the left there is the Raman spectrum over the range from 100 to 3500 cm^{-1} and on the right the single channel spectrum between 5894 and 9294 cm^{-1}. Notice that the spike at 9394 cm^{-1} is due to the laser line.

10.10 Smooth

In OPUS the Savitzky–Golay algorithm [1–3] is applied to smooth a spectrum. Possible values for smoothing points are 5 to 25. Open the smooth dialog box shown in Fig. 10.26, select the spectrum as usual, choose the number of smoothing points and click on the *Smooth* button to start the function.

After a spectrum is smoothed it becomes similar to the result of an experiment obtained at a lower resolution. Thus smoothing has a cosmetic effect on the spectrum; it reduces the noise but also distorts the spectral features. Figure 10.27 depicts the IR spectrum of ibuprofen and shows the effect on the data of smoothing with 9 smoothing points. Obviously, the original spectrum always shows more pronounced peaks than the smoothed one.

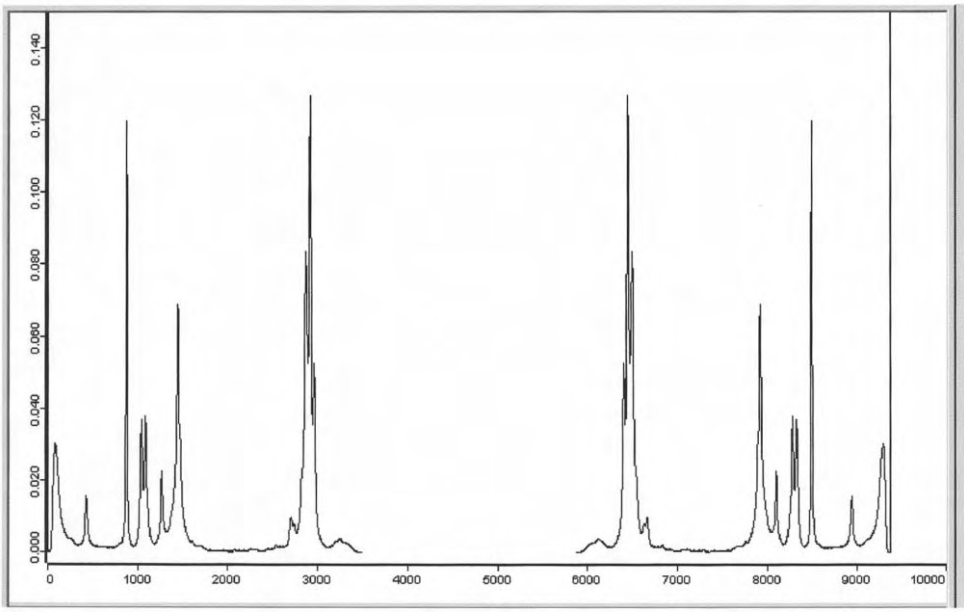


Figure 10.25. The Raman spectrum and single channel spectrum of ethanol.

Figure 10.26.
The *Smooth* dialog box.

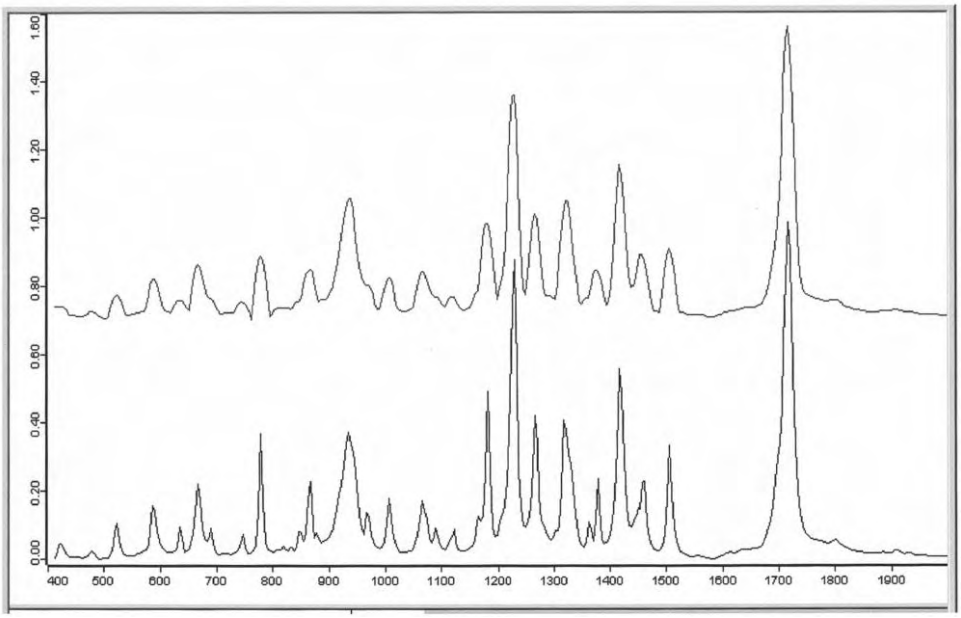


Figure 10.27. The IR spectrum of ibuprofen: bottom original spectrum; top spectrum smoothed with 9 smoothing points.

10.11 Derivative

This function calculates the first- to fifth-order derivative of a spectrum applying the Savitzky–Golay algorithm. This allows a simultaneous smoothing of the spectrum, which reduces the noise generated by the derivation. The minimum number of smoothing points depends on the order of the derivative. A maximum of 25 points is allowed. Enter the spectrum file into the derivative dialog box (see Fig. 10.28), choose the number of smoothing points and the order of the derivative, and finally click on the *Process* button to start the function.

The result is an additional data block named "1.Der.", "2.Der." or "*n*.Der." (for an order of 3 or higher), which is added to the file data block list (see Fig.10.29).

The order of derivative is stored in the data block; use the *Show Parameter* command to display the content of the block.

The derivative of a spectrum can be further differentiated. For example, the result of taking the derivative of a first order derivative will be a second order derivative.

Figure 10.30 demonstrates the usefulness of a spectrum derivative. It is obvious that the band at the top is an overlapping of two neighbouring bands, but it is not so easy to determine the exact peak position of the right band at

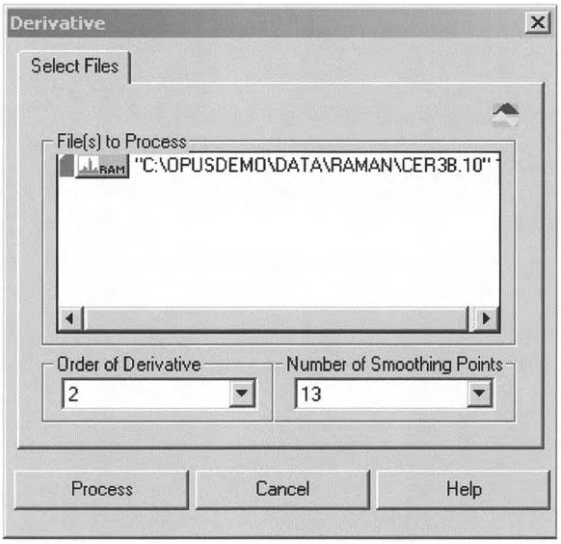


Figure 10.28.
The *Derivative* dialog box.

Figure 10.29. The *Derivative* icons.

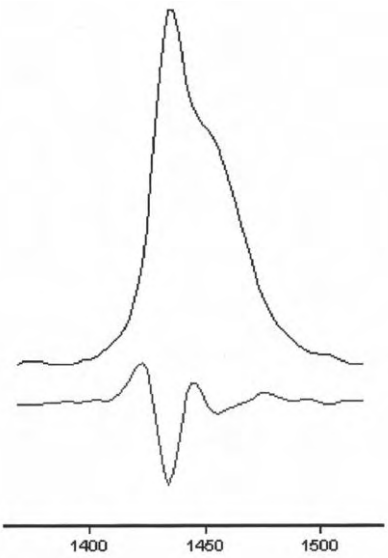


Figure 10.30. An example of a spectrum derivative: top, original band; bottom, second derivative.

about 1450 cm^{-1}. Here the second derivative is helpful: the positions of the minima provide the peak positions demanded.

10.12 Wavenumber Calibration

This function re-calibrates the x-axis of a spectrum including any peak pick lists. The new axis and new wavenumbers are calculated by using the two parameters M and A. The formula is:

$$\tilde{\nu}_{new} = M\tilde{\nu}_{old} + A \tag{10.21}$$

Open the wavenumber (frequency) calibration dialog box (see Fig. 10.31), select the spectrum as usual, enter the multiplication factor M and the offset A, and then click on the *Calibrate* button to start the function.

After the calibration the parameters M and A can be found in the spectrum data block. This gives you the possibility to revoke the calibration.

Examples of the calibration are depicted in Fig. 10.32, where the original spectrum is the Raman spectrum in the spectral range 700–1800 cm^{-1}. The original data set before the calibration is listed in Fig. 10.33. After the calibration the wavenumbers have changed, as evidenced by the wavenumbers of the first and last data points, see Fig. 10.34. Furthermore, two additional parameters "Mult. for Freq. Calib." and "Add for Freq. Calib." have been included in the list.

The x-axis of a spectrum can be modified several times. The parameters M_{total} and A_{total}, representing the entire changes in comparison to the original spectrum, then appear in the data parameter list. These changes can be reversed in just one step.

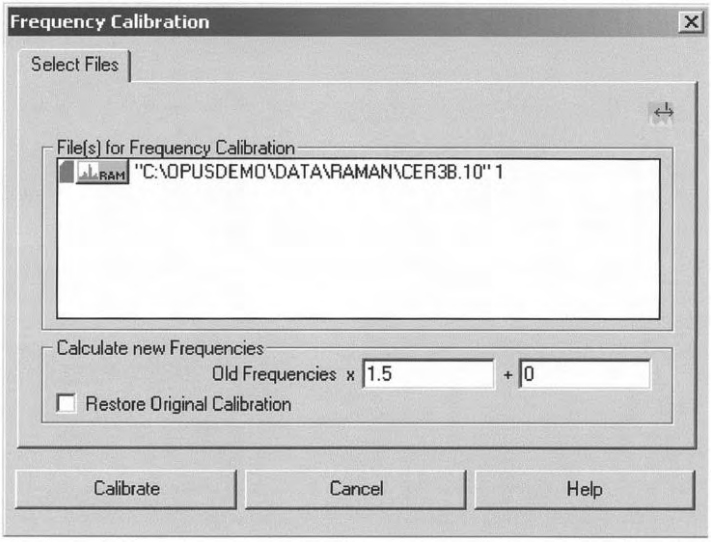
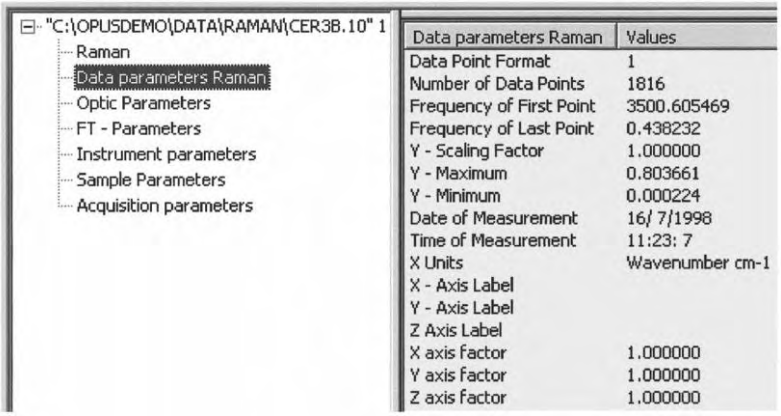


Figure 10.31. The *Frequency Calibration* dialog box.



Data parameters Raman	Values
Data Point Format	1
Number of Data Points	1816
Frequency of First Point	3500.605469
Frequency of Last Point	0.438232
Y - Scaling Factor	1.000000
Y - Maximum	0.803661
Y - Minimum	0.000224
Date of Measurement	16/ 7/1998
Time of Measurement	11:23: 7
X Units	Wavenumber cm-1
X - Axis Label	
Y - Axis Label	
Z Axis Label	
X axis factor	1.000000
Y axis factor	1.000000
Z axis factor	1.000000

Figure 10.32. An original set of data parameters before the calibration.

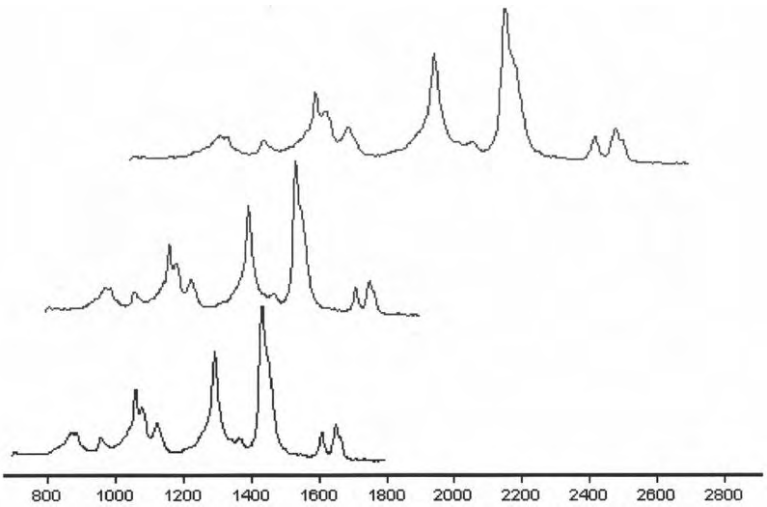


Figure 10.33. Examples of frequency calibration: bottom, original spectrum; middle, spectrum calibrated with $M = 1$ and $A = 50$; top, spectrum calibrated with $M = 1.5$ and $A = 0$.

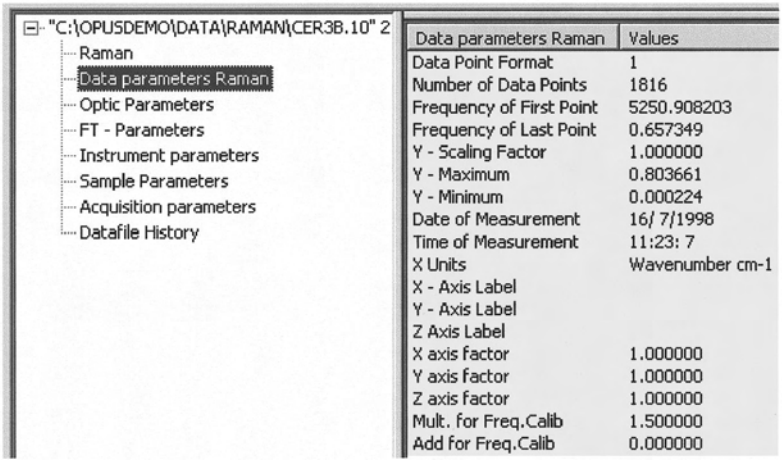


Figure 10.34. Set of data parameters after calibration with $M = 1.5$ and $A = 0$.

10.13 Raman Correction

The special features of the optics and the frequency dependent scattering, which occur in a Raman spectrum, can be reduced to a high degree by using this correction. There is an undo function for this correction. The file selection is limited to Raman spectra.

Select a file on the first page of the Raman correction dialog box, define the correction method, and indicate the reference spectrum that is to be used as well as the temperature on the second page (Fig. 10.35). Start the correction by clicking on *Correc*t. The applied corrections can be undone with the *Restore Original Data* option. Along with the correction two flags are set, that can be seen in the *Instrument parameters* field shown in Fig. 10.36. This avoids using the same correction more than once. Ensure that the wavenumber of the Raman laser and the temperature of the lamp have not changed right before performing the correction.

The theoretical background for the *Scatter Correction* is that the scattering intensity is a function of the wavenumber (Rayleigh's ν^4-law, see Section 5.7). The effect increases with the spectral distance of the line of interest from the wavenumber of the excitation laser. To correct this the Raman intensity data are multiplied point-by-point by:

$$\left(\frac{\tilde{\nu}_{\text{Laser}}}{\tilde{\nu}}\right)^4 \tag{10.22}$$

By checking the *Reference Correction* box the effects of the spectrometer optics can be corrected for. This requires a spectrum of a tungsten lamp for calibration.

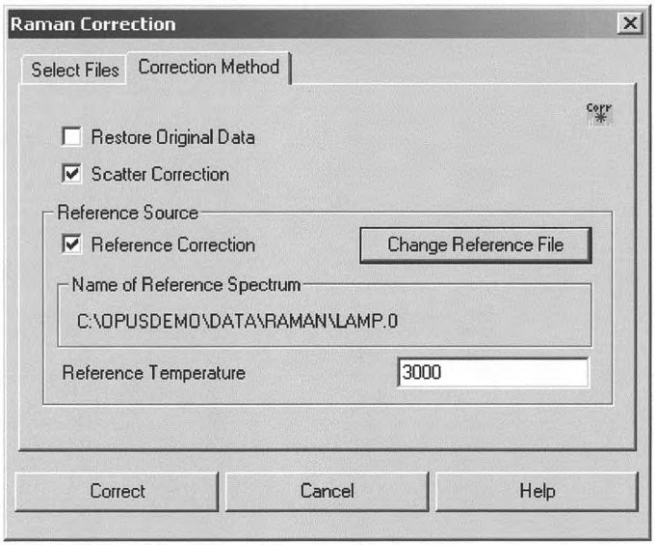


Figure 10.35. The *Raman Correction* dialog box: second page.

Instrument parameters	Values
High Folding Limit	15798.000000
Low Folding Limit	0.000000
Number of Sample Scans	400
Scan time (sec)	697.679174
Running Sample Number	1342
Peak Amplitude	-463
Peak Location	7084
Sample Spacing Divisor	1
Instrument Type	RFS100
Focal Length	125.000000
Absolute Peak Pos in Laser*2	42210
Laser Wavenumber	15798.000000
Raman Laser Wavenumber	9394.000000
Raman Laser Power in mW	560
Ready Check	ON
Sample Spacing Multiplicator	1
Number of Good FW Scans	200
Number of Good BW Scans	200
Number of Bad FW Scans	3
Number of Bad BW Scans	3
Backward Peak Amplitude	-478
Backward Peak Location	7134
Actual Signal Gain	1
Number of Background Scans	0
Raman Background Corrected	YES
Calibration Lamp Spectrum Path	C:\OPUSDEMO\DATA\RAMAN
Calibration Lamp Spectrum	LAMP.0
Temp. of Calibration Lamp	3000.000000
Raman Scattering Corrected	YES

Figure 10.36. List of instrument parameters with Raman correction flags added.

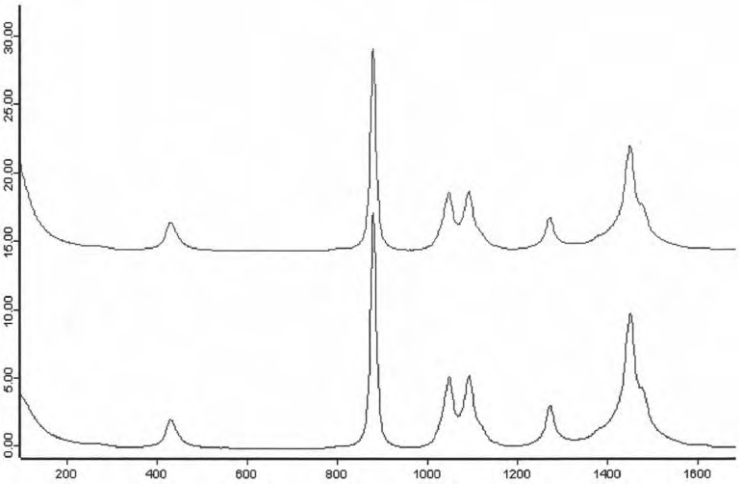


Figure 10.37. An example of Raman correction: spectrum of ethanol in the spectral range 100–1600 cm^{-1}; top, no correction; bottom, with background and scatter correction.

This spectrum should not be more than 2 weeks old to reflect the aging of the lamp. The name of the spectrum to be used can be specified *(Change Reference File)*. In the case of *Reference Correction*, the intensity data are multiplied point-by-point as follows:

(blackbody spectrum)(Raman spectrum)/(reference spectrum).

An example of the Raman correction is given in Fig. 10.37.

10.14 Blackbody

This function calculates the spectral radiance of a blackbody:

$$L(\tilde{\nu}) = 2\times10^8 hc^2\tilde{\nu}^3\left[\frac{1}{\exp(100hc\tilde{\nu}/kT)-1}\right] \tag{10.23}$$

where

$c = 2.99792458 \times 10^8$ m s^{-1}
$h = 6.626176 \times 10^{-34}$ J s
$k = 1.380662 \times 10^{-23}$ J K^{-1}

Because vibrational spectra are mostly presented on the wavenumber scale, the spectral radiance is here expressed as intensity per unit solid angle in the wavenumber interval between $\tilde{\nu}$ and $\tilde{\nu}$ + d$\tilde{\nu}$.

The wavenumber region and data point spacing, as well as the units of the *x*-axes will be taken from a single channel spectrum that is entered in the black-

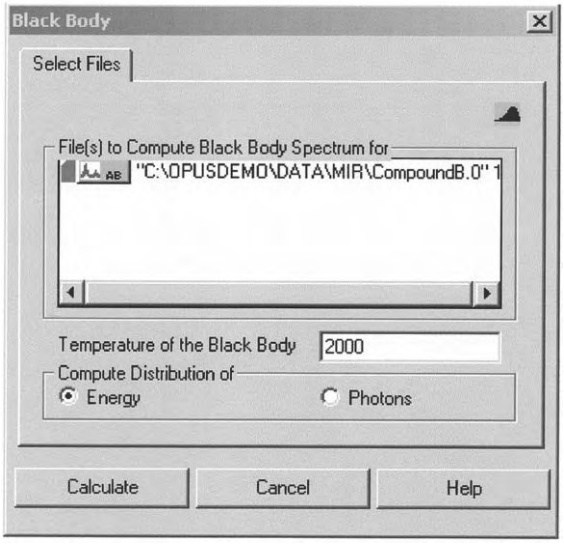


Figure 10.38.
The *Blackbody* dialog box.

body dialog box (see Fig.10.38). The temperature T of the light source will be given in K and is stored together with the computed distribution. The computed distribution is saved as single channel data block.

Figure 10.39 shows the radiance distribution of a blackbody calculated for $T = 1000$, 1500, and 2000 K, where, in order to cover the spectral range from 400 to 10000 cm^{-1}, two files have been selected, namely an MIR spectrum (400–4000 cm^{-1}) and an NIR spectrum (4000–10000 cm^{-1}).

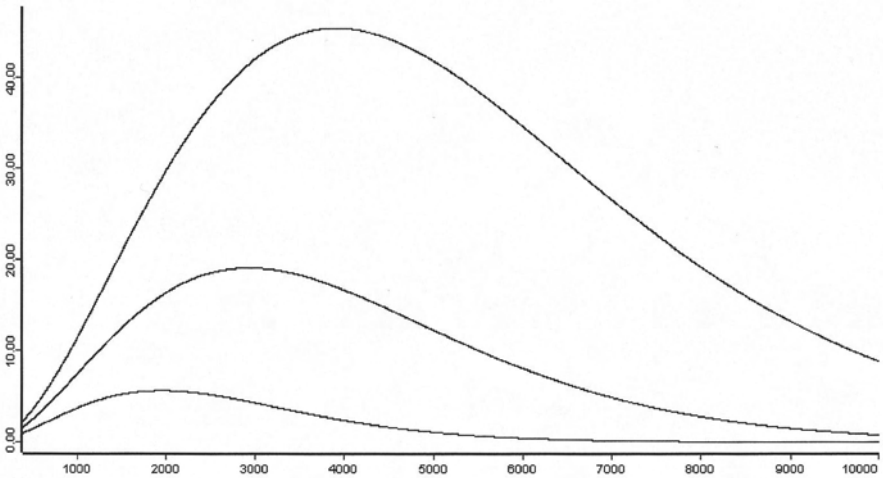


Figure 10.39. The spectral radiance of a blackbody at various temperatures; from top to bottom 2000, 1500, and 1000 K.

When Raman spectra are chosen, the *x*-axis is shifted and switched so that the Raman laser signal is at zero and the Stokes region of the spectrum is in positive wavenumbers. The Raman laser frequency is taken from the parameter "*RLW*" of the experimentally determined spectrum or can be set manually.

As can be seen in the dialog box, it is also possible to calculate the photon flux versus wavenumber.

10.15 Conversion from Interferogram to Spectrum

This function calculates a spectrum from an interferogram and thereby performs the same steps that are usually done immediately after data acquisition, namely:

- Apodization,
- Phase computation,
- Zerofilling,
- Fourier transformation of the interferogram, and
- Phase correction.

This procedure is helpful for transforming interferograms after measurement by allowing different parameters to be used for apodization, phase correction and zerofilling. Utilizing this procedure you can "play" with the Fourier transformation.

The file entered on the first page of the appropriate dialog box has to be an interferogram (see Fig. 10.40). The data should lie between the *High folding*

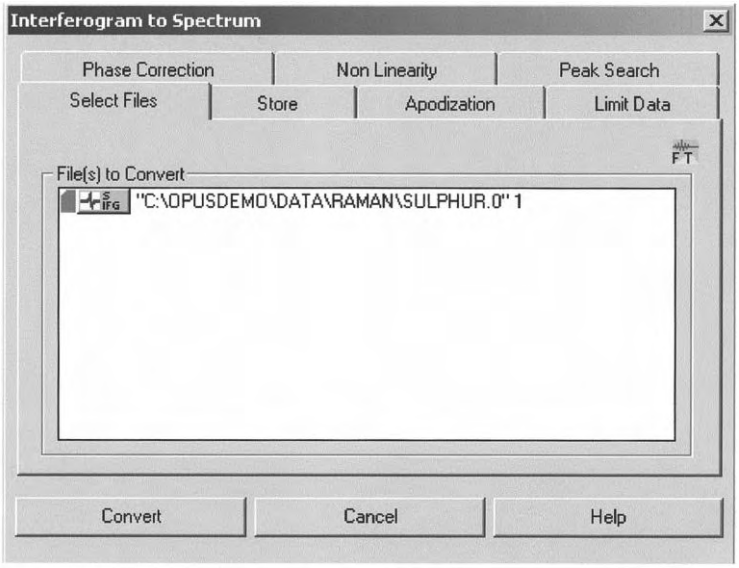


Figure 10.40. The *Interferogram to Spectrum* dialog box: *Select Files* page.

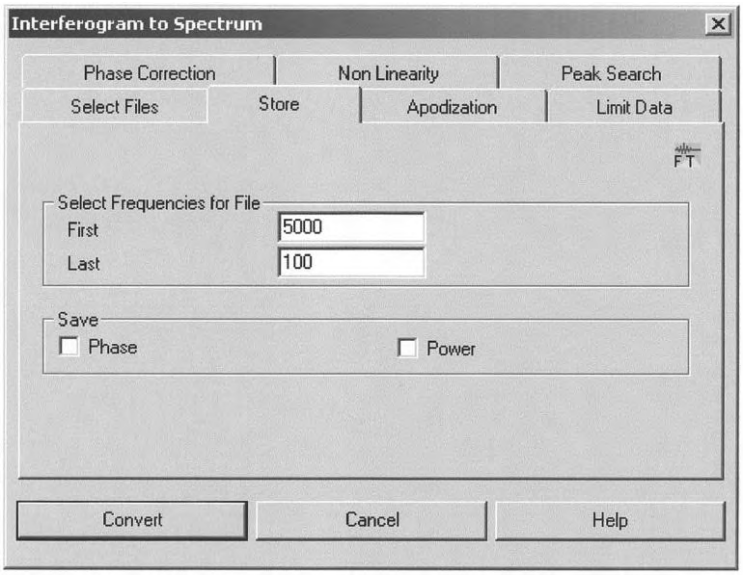


Figure 10.41. The *Interferogram to Spectrum* dialog box: *Store* page.

limit and the *Low folding limit* parameters used during the measurement; these parameters can be obtained from the instrument parameter block of the interferogram.

On the *Store* page (Fig. 10.41) specify the wavenumber range and the spectrum type to be saved. Notice that the calculated spectrum block always is of type "Single-Channel"! In addition, one can also save the Phase spectrum and the Power spectrum, which can be calculated from the parts of the interferogram known from forward and backward scans with the *Phase Resolution* setting.

To reduce the artifacts due to the discrete Fourier transformation (see also Chapter 5) choose an appropriate function on the *Apodization* page depicted in Fig. 10.42. For standard measurements of liquid or solid samples, the *Blackman–Harris-3-term* is recommended. To obtain the highest resolution, choose no apodization *(Boxcar)* or if necessary a weak apodization *(Norton–Beer-wea*k).

Zerofilling is the adding of zeros to both ends of the interferogram before the Fourier transformation. It results in an increased number of points in the spectrum, which corresponds to an interpolation. Due to zerofilling the number of spectrum points can be enlarged by the specified factor (2, 4, 8…512). The more data points in the spectrum, the better-looking the sharp lines become (cosmetic effect). However, this requires n-times the computing time and n-times the storage space.

For single-sided interferograms a minimum factor of 2 is required. For double-sided interferograms, the zerofilling factor can be halved in comparison to single-sided collections.

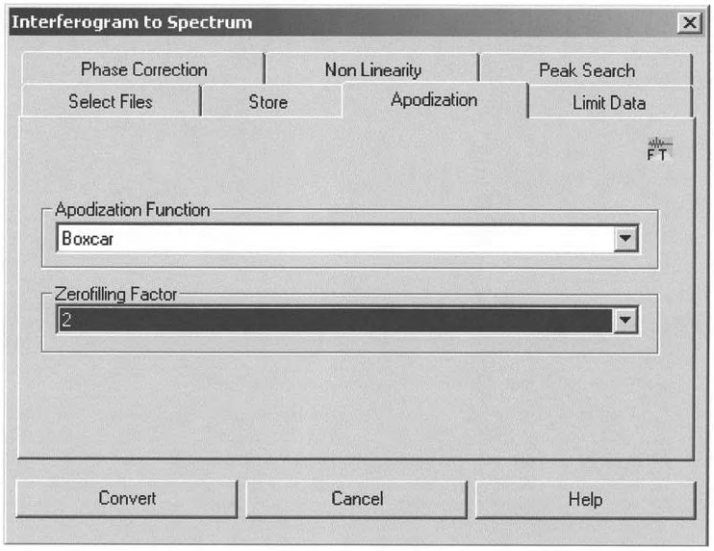


Figure 10.42. The *Interferogram to Spectrum* dialog box: *Apodization* page.

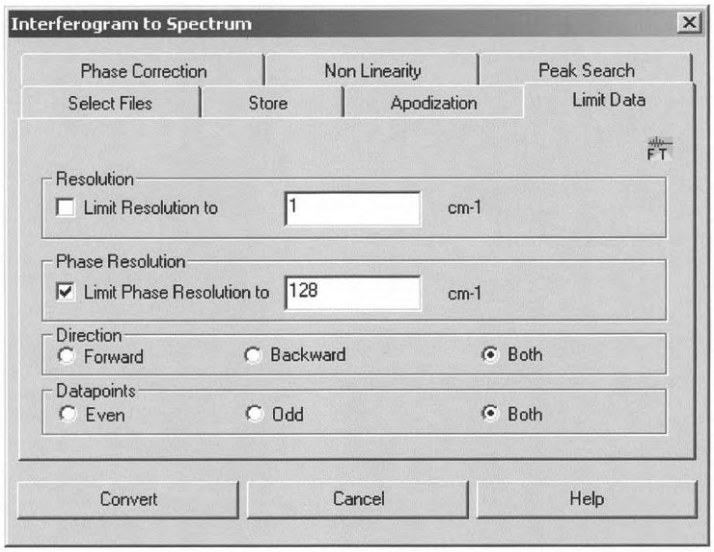


Figure 10.43. The *Interferogram to Spectrum* dialog box: *Limit Data* page.

If the box *Limit Resolution* on the *Limit Data* page (Fig. 10.43) is checked, one can vary the resolution by entering a "Resolution Limit" that is larger than or at least equal to the value used in the measurement. Then only a fraction of the measured interferogram is used.

The *Phase Resolution* is the resolution for computing the phase. Normally the same resolution as was used for the measurement should be used.

Interferograms recorded in forward/backward mode can be processed using either the scans during the forward or the backward movement of the mirror. If the two directions of the mirror travel should be evaluated, the forward and the backward scan will be transformed separately, followed by a phase correction and calculation of the average spectrum.

If the data have been recorded in multiplex mode, then the interferogram contains alternating data from two ADCs. With the options *even* and *odd* the data from both ADCs can be evaluated separately. There is no such option for backward scans. Therefore, multiplex measurements should be recorded only with pure *Forward* modes.

By selecting *even* the data indexed by even numbers I_0, I_2 ... will be transformed, while *odd* transforms data indexed by odd numbers I_1, I_3 ...

The *Phase Correction* (see Fig. 10.44) can be thought of as symmetrizing the interferogram, which is always necessary due to the asymmetry of any measured interferogram. Several phase correction methods are available:

- *Mertz*: This is the standard procedure.
- *Mertz signe*d: The modified Mertz function is used, if the single channel spectrum is expected to contain negative contributions.
- *Power spectru*m: This can be used instead of Mertz or Foreman, but only for double-sided interferograms, when the spectrum has wide ranges of low

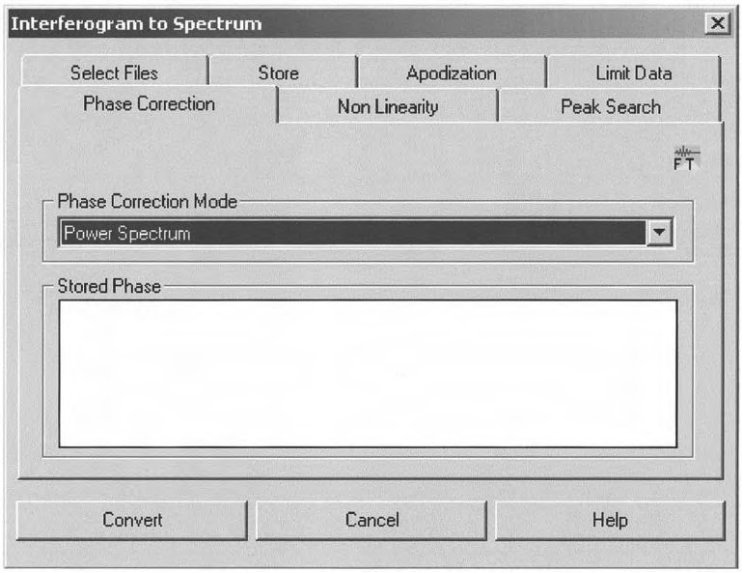


Figure 10.44. The *Interferogram to Spectrum* dialog box: *Phase Correction* page.

light intensity (e.g. total absorption, Raman, emission). The disadvantage is that the noise increases by a factor of up to $\sqrt{2}$ compared with that of the Mertz or Forman procedures.

- *Mertz/Stored Phase*: Like Mertz, but the phase will not be calculated. Instead the phase will be taken from previously existing data for which the phase was obtained using the regular Mertz method. This can be useful for example, if the maximum of the interferogram is not very well defined, as e.g. for emission or Raman measurements. This method is also useful, if the spectra are expected to contain parts with negative contributions. In this case, the phase information used should be taken from a spectrum with only positive values.
- *No/Save complex data*: The data will not be phase corrected, but is instead transformed in complex form and stored as with real and imaginary parts.
- *Forman*: A method mathematically equivalent to Mertz, offers a slightly higher precision at the cost of higher computational costs.

On the *Peak Search* page shown in Fig. 10.45 you have the option of how to select the position of zero path difference (ZPD):

- *Absolute largest value* finds the peak with the highest absolute intensity.
- *Maximum* finds the highest peak with the largest positive value.
- *Minimum* looks for the peak with the largest negative value.
- *Mid between Min./Max* calculates a value halfway between the minimum and the maximum.

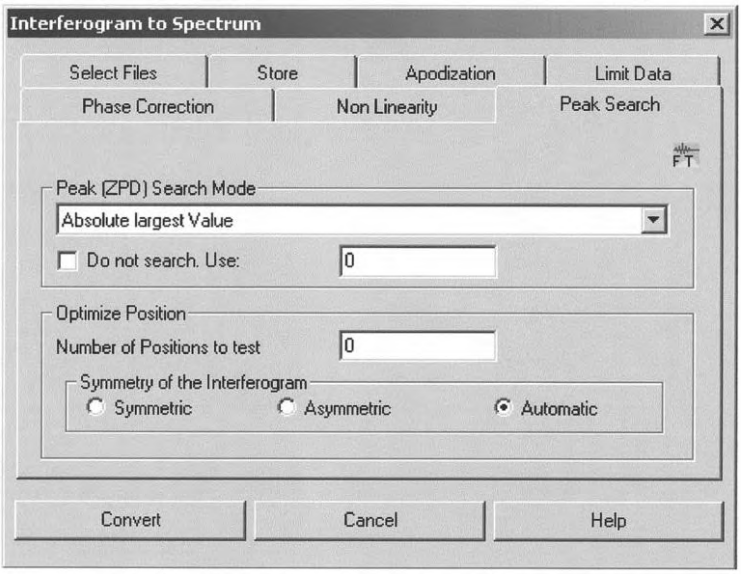


Figure 10.45. The *Interferogram to Spectrum* dialog box: *Peak Search* page showing zero path difference.

- *No peak search* uses the position saved in the interferogram; if this value is known, it can also be entered manually.

Including data points in addition to those chosen by the algorithm can influence the position of the reference point. Every point will be tested for its symmetry; the point with the highest symmetry will be chosen as the ZPD. Whether or not symmetry or antisymmetry is to be tested, can also be specified.

Figure 10.46 represents the Raman spectra of sulphur calculated by Fourier transformation of the interferogram of the file SULPHUR using no apodization (boxcar) and the apodization function Norton–Beer weak, respectively, a zerofilling factor of 2, and the power spectrum for the phase correction. See if you obtain the same spectra.

Keep in mind that the transformation interferogram to spectrum always results in a single channel spectrum. Therefore, a calculated spectrum must be converted in a respective way.

Choose other apodization functions and elucidate their influence on spectral lines.

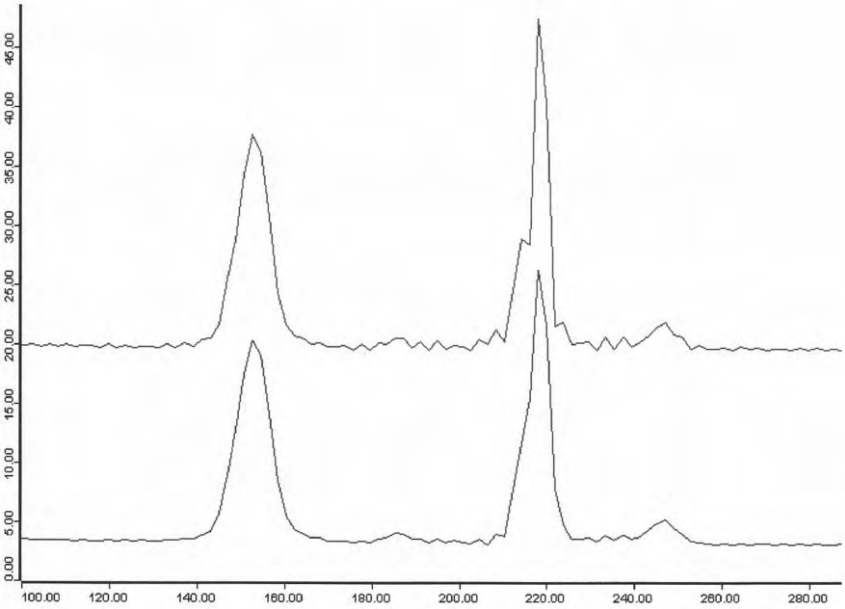


Figure 10.46. The Raman spectrum of sulphur in the spectral range 100–280 cm^{-1} calculated by Fourier transformation of the interferogram: top, no apodization (boxcar); bottom, apodization function Norton–Beer weak. In both cases, a zerofilling factor of 2 and the power spectrum for phase correction were chosen. Further parameter used: Store page: selected frequencies for file first 9394 and last 5894; Limit data page: limit resolution to 4 cm^{-1}, limit phase resolution to 32 cm^{-1}, direction both, data points both; Peak search page: mode absolute largest value, symmetry of the interferogram automatic.

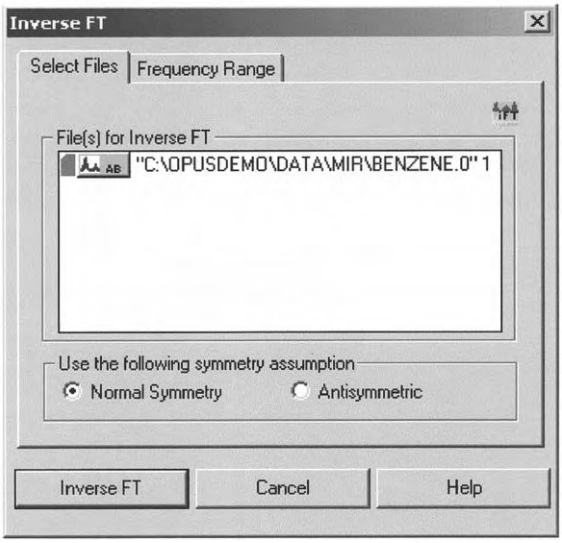


Figure 10.47. The *Inverse Fourier Transformation* dialog box.

10.16 Inverse Fourier Transformation

This function transforms a spectrum back to an interferogram. It can be used with all types of spectra. You have to specify whether the measured interferogram was originally a symmetric or an antisymmetric one. In most cases you should choose a symmetric transformation, because most of the spectra were obtained involving a phase correction. The antisymmetric inverse Fourier transformation should only be applied to complex spectra, e.g. spectra also containing the imaginary part. Notice the interferogram generated in this manner comprises only values on the positive *x*-axis.

Select the file and wavenumber range as usual in the dialog box (Fig. 10.47) and specify the symmetry as normal or antisymmetric. Start the processing by clicking on the *Inverse FT* button.

10.17 Post Zerofilling

By using post zerofilling you can increase the number of points in the spectrum, thus corresponding to an interpolation. This yields better line shapes (smoother) for spectra measured with high resolution. During the Fourier transformation a zerofilling is automatically carried out over the whole spectral range. However, this increases the total size of the data file. Using post zerofilling allows you to apply the interpolation once again for only the frequency range of interest, which keeps the size of your files small. You have to specify the

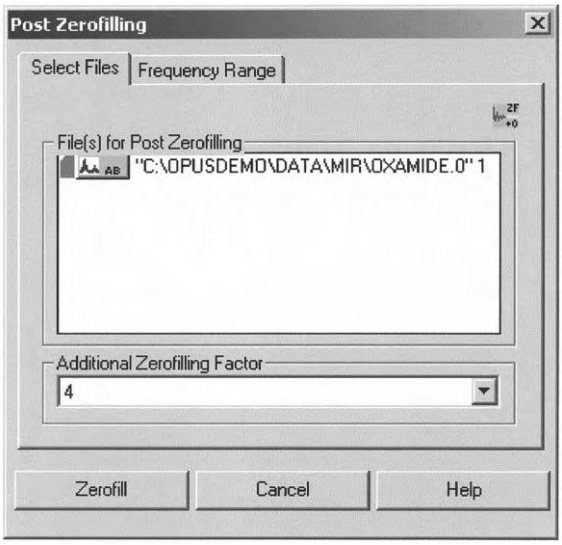


Figure 10.48. The *Post Zerofilling* dialog box.

zerofilling factor, i.e. a factor ranging from 2 to 512. A zerofilling using a factor of 2, for example will double the number of spectrum points by interpolation. However, zerofilling should not be confused with an increase in spectral resolution.

Specify the file on the first page of the dialog box (Fig. 10.48) and select, on the second page of the dialog, the spectral range that will be processed.

Let us consider two cases:

If a spectrum with a spectral resolution of 8 cm^{-1} is post zerofilled with an additional zerofilling factor of 2, the digital resolution after the interpolation is 4 cm^{-1}.

If a spectrum with a spectral resolution of 8 cm^{-1} is post zerofilled with an additional zerofilling factor of 8, the digital resolution after the interpolation is 1 cm^{-1}.

Note that, in both cases, however, the spectral resolution is still 8 cm^{-1}. To avoid artifacts allow at least 50 more data points on each side of the desired interval. This region should also contain meaningful spectral information.

In the example given in Fig. 10.49 a post zerofilling factor of 4 was used.

10.18 Fourier Self Deconvolution

This option performs a Fourier Self Deconvolution (FSD) of a spectrum. The aim of this operation is to enhance the apparent resolution of a spectrum or to decrease the width of all lines contributing to the spectral range under

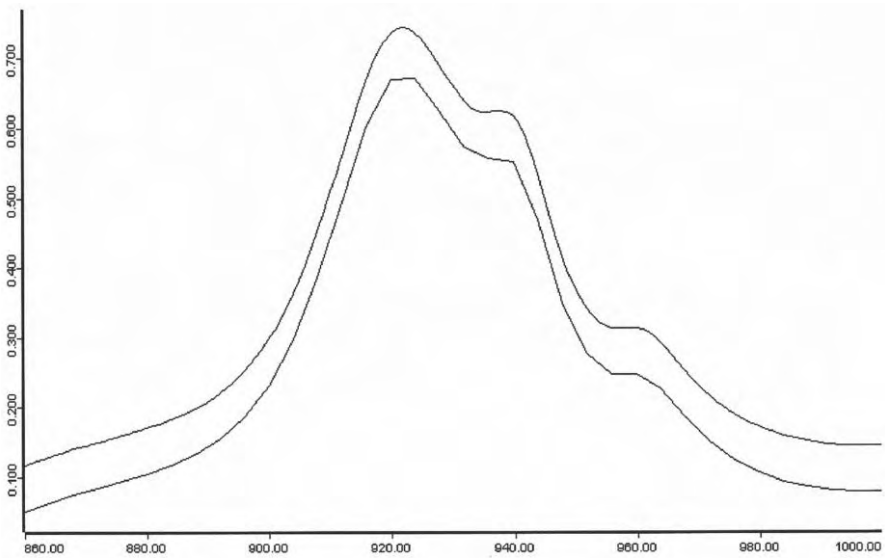


Figure 10.49. The digital resolution before and after *Post Zerofilling:* bottom, original band; top, after processing with zerofilling factor of 4.

investigation. This assists in resolving a spectral range comprising overlapping broad lines into narrower lines. Using FSD allows you to narrow the line width of broad envelopes in order to improve a separation of peaks and shoulders.

Notice this function is only suited for envelopes much broader than the spectral resolution.

The type of the line-broadening function (LBF) may be either Lorentzian or Gaussian. The appropriate type depends on the nature of the line broadening mechanism. If in doubt it is recommended to start with a Lorentzian shape. After you have selected a file and wavenumber range as usual in the dialog box (Fig. 10.50), specify on the *Adjust Parameter* page a deconvolution factor and a suppression factor for the noise, or alternatively, a factor for the bandwidth and the resolution enhancement. Start the function using the *Deconvolute* button.

FSD assumes that the experimental spectrum consists of well-resolved narrow peaks, which have been convoluted with the same sort of LBF. If the shape and width of this LBF are known, its effect can be mathematically removed from the spectrum. This is done in the interferogram space where removal of the LBF simply corresponds to multiplication by a "deconvolution function" being the inverse of the Fourier transformation of the LBF.

In other words, the deconvolution corresponds to a multiplication of the interferogram $I(x)$ by the deconvolution function $\exp(ax)$ for a Lorentzian shape and $\exp(ax^2)$ in the case of a Gaussian shape. The deconvolution factor is the maximum value of this function at the end of the x-interval used.

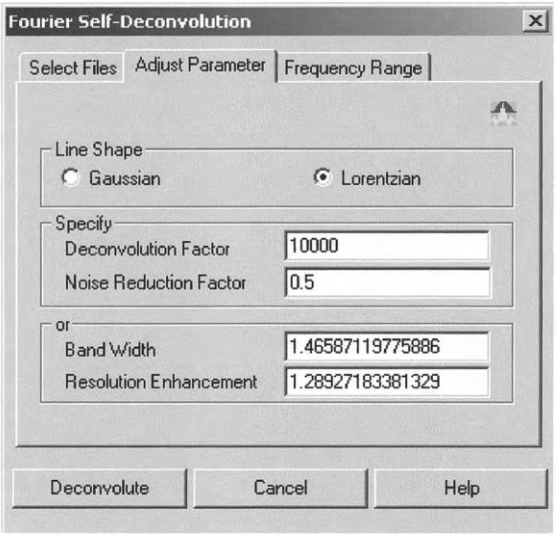


Figure 10.50. The *Fourier Self Deconvolution* dialog box.

The amplification of the interferogram by the deconvolution also amplifies the noise, especially at the wings of the interferogram where the signal-to-noise ratio is worse than in the range of the central peak. To reduce the noise, an apodization with a Blackman–Harris function is always performed at the same time, such that the interferogram is multiplied by the product of an ascending deconvolution function and a descending apodization function. The product of both functions is 1.0 at the zero path difference point and zero at the end of the interval. It exhibits a maximum between these boundaries. Its maximum value determines the maximum net amplification of the interferogram.

A deconvolution factor of 100, 1000, and 5000 corresponds to a maximum net amplification of 3.4, 12.8, and 40 for Lorentzian line shapes and 1.06, 3.2, and 16 for Gaussian line shapes, respectively. In the case of the Lorentzian it is recommended to try deconvolution factors of the order 50, 100, 1000, and 5000 or to stop, if over-deconvolution occurs, i.e. the resultant spectrum shows artificial oscillations.

The noise reduction factor should range from 0.0 to 1.0. It is the fraction of the interferogram to which the combination of deconvolution and apodization is applied. A value of 1.0 corresponds to the full interferogram. It is recommend to start with a value of 0.5, i.e.the half-length of the interferogram. If the spectrum has been calculated using a zerofilling factor larger then 2, then a start value of 1 / (zerofilling factor) is recommended.

Only spectral ranges with signals of comparable line width should be selected. Broad signals cannot be deconvoluted if there are sharp signals in the same range. At the boundaries of the selected spectral range the intensities should

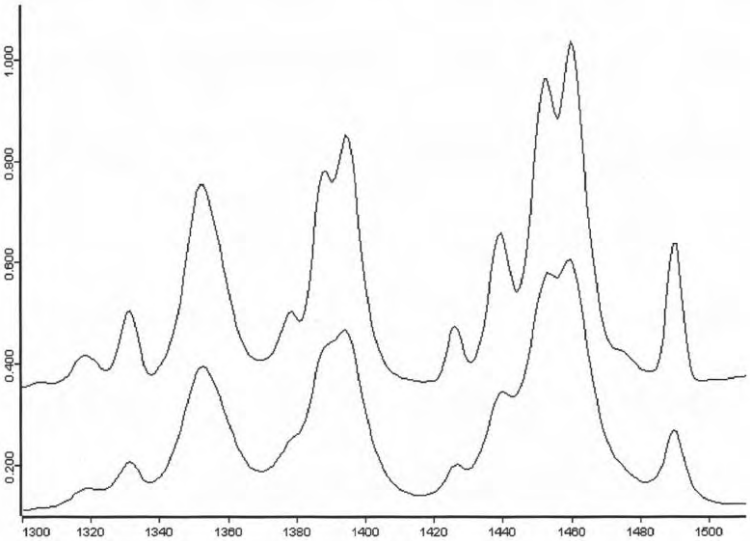


Figure 10.51. The IR spectrum of the compound B in the spectral range 1300–1500 cm^{-1} before and after *Fourier Self Deconvolution:* bottom, Original spectrum; top, after processing using a deconvolution parameter of 10000 and a noise reduction factor of 0.5.

be close to zero. If this is not the case, the spectrum should be baseline corrected prior to deconvolution.

Figure 10.51 shows a portion of the IR spectrum of the compound B before and after a deconvolution, which has been performed using a deconvolution parameter of 10000 and a noise reduction factor of 0.5. It is evident, for the deconvoluted spectrum, that the intensity at the peak maximum position is higher than that of the original spectrum. However, the peak ratios are not changed.

10.19 Symmetric Fourier Transformation

The symmetric Fourier transformation is used if a phase correction is not necessary, because the data contains one half of a symmetric or antisymmetric interferogram. This may occur for example, if the interferogram is generated by an inverse FT.

Again, it is advised that, if the interferogram is perfectly symmetric or perfectly antisymmetric, you should select the symmetric transformation. Define the data range used for the transformation on the *Frequency Range* page and start the transformation. The result is a single channel spectrum. In the case of an antisymmetric transformation only the real part of the single channel spectrum will be saved.

10.20 Abscissa Conversion

This function changes the x-axis units of a spectrum. The wavenumber units are converted to wavelength units, micrometer or nanometer, and vice versa according to the formula:

$$\text{wavelength [µm] wavenumber [cm}^{-1}\text{]} = 10000 \tag{10.24}$$

The relevant dialog box shown in Fig. 10.52 comprises three pages. On the first page, the spectra to be processed are selected as usual. The conversion direction can be selected in the box on the right-hand side of this page. On the second page (Fig. 10.53), the spectral range can either be defined by the user or can be taken from the original spectrum.

Because a digitally recorded spectrum consists of equidistant data points, e.g. two data points per wavenumber, after the conversion to wavelengths the data points are no longer equidistant due to the hyperbolic conversion function. This is exemplary listed in Tab. 10.1. Since OPUS saves spectra as a set of equidistant data points only, an interpolation must be performed after the abscissa conversion.

The kind of interpolation can be determined in two different ways:

1. If fixed wavenumber limits are chosen, you can define the number of data points in the resultant spectrum. To illustrate this procedure, the IR spectra of polystyrene in the spectral range from 2750 to 3250 cm^{-1} (4 cm^{-1} resolution, 126 data points) converted with differently chosen data points (63,

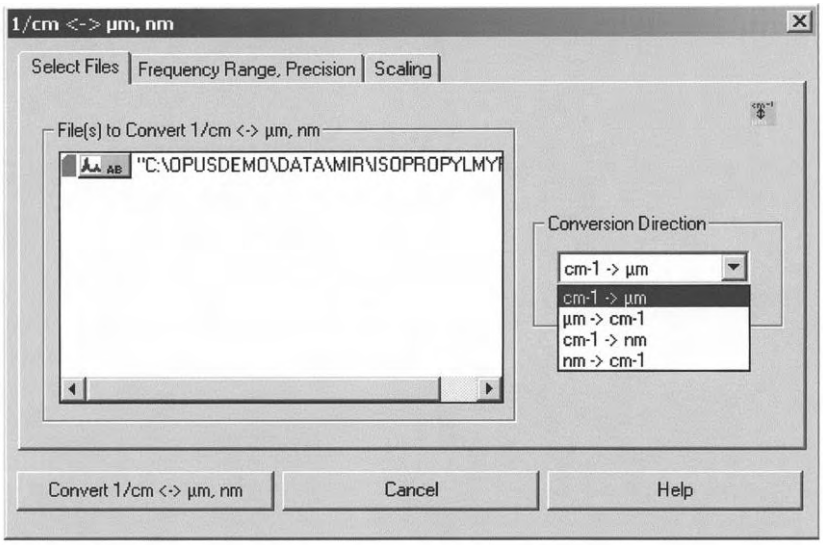


Figure 10.52. The abscissa conversion dialog box: first page.

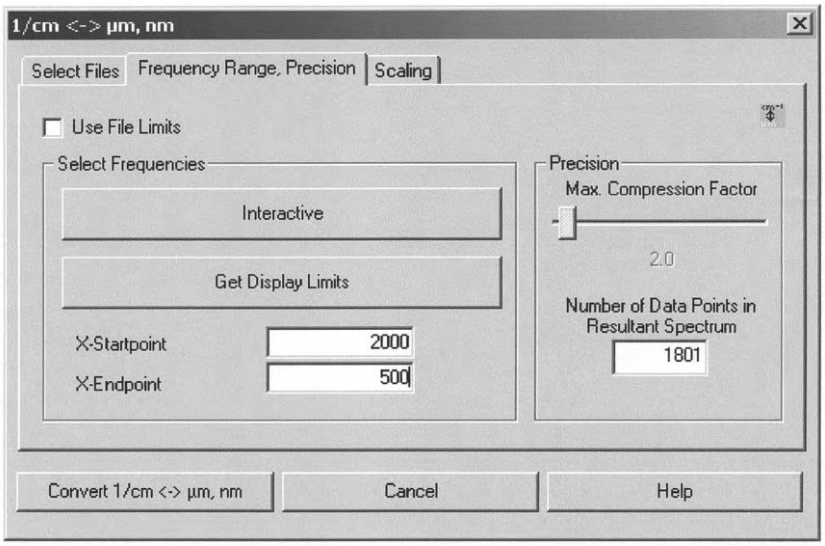


Figure 10.53. The abscissa conversion dialog box: second page.

Table 10.1. Example of conversion from wavenumber to wavelength

Wavenumber [cm^{-1}]	Wavelength [µm]	Difference [µm]
400	25.00	
		1.19
420	23.81	
		1.08
440	22.73	
		0.99
460	21.74	
		0.91
480	20.83	
		0.83
500	20.00	

126, 251, and 501) are depicted in Fig. 10.54. As you can see, the "wavelength" spectrum with 63 data points exhibits strong distortions.

2. If *Use File Limits* is checked, the *Maximum Compression Factor* (MCF) regulates the interpolation.

The minimum number of data points for the created "wavelength" spectrum is equal to the number of data points found in the input "wavenumber" spectrum. The MCF defines the conversion properties in the high wavenumber end of the "wavenumber" spectrum, which is converted to the low wavelength end of the "wavelength" spectrum.

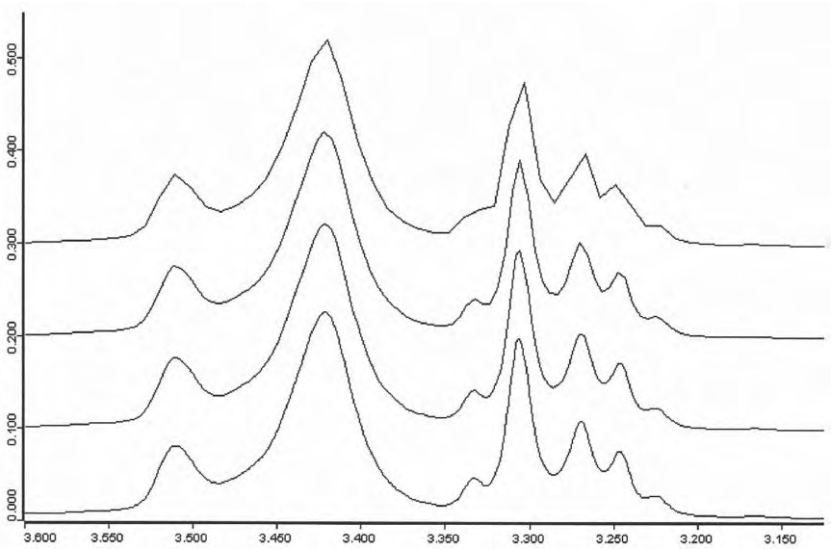


Figure 10.54. The IR spectra of polystyrene in the wavenumber range from 2750 to 3250 cm^{-1} converted with differently chosen data points: from top to bottom 63, 126, 251, and 501. Note the unit of the *x*-axis is μm.

If for instance an MCF of 5 is chosen, five wavenumber intervals (spacing between data points) are converted to one wavelength interval and therefore spectral information is lost. The conversion properties get better when lower wavenumbers (higher wavelengths) are considered. The MCF can be varied between 0.5 and 50, but the new spectrum never has fewer data points than the original spectrum. Hence, it can occur that the number of data points does not change for a factor larger than for instance 10.

Using a small MCF the number of data points of the new spectrum may become rather large. This depends on the start and end wavenumber and the spectral interval of the original spectrum. By back-transformation of the "wavelength" spectrum and comparison with the original spectrum a reasonable factor can be decided.

How the chosen MCF affects the spectral feature is again demonstrated in the IR spectrum of polystyrene shown in Fig. 10.55.

On the third page, the scaling of the intensities can be adjusted (see Fig. 10.56). When you select *Preserve y-Value*s, the intensities will be taken from the original spectrum, but effects of interpolation can appear. The spectrum generally looks the same, however, with a significantly changed *x*-axis.

Using *Preserve Integrals* has the effect of multiplying the y-values by a frequency- or wavelength-dependent factor (proportional to $1/x^2$), so that integrating the original spectrum and the new spectrum within the same limits yields the same sum.

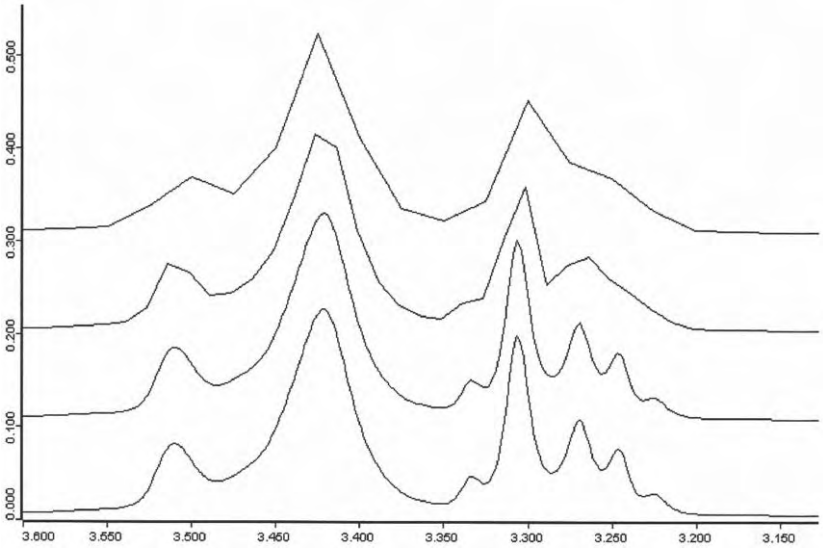


Figure 10.55. The IR spectra of polystyrene in the wavenumber range from 2750 to 3250 cm^{-1} converted using file limits and different *Maximum Compression Factors* (MCF): from top to bottom MCF = 10, 5, 1, and 0.5. Note the unit of the *x*-axis is µm.

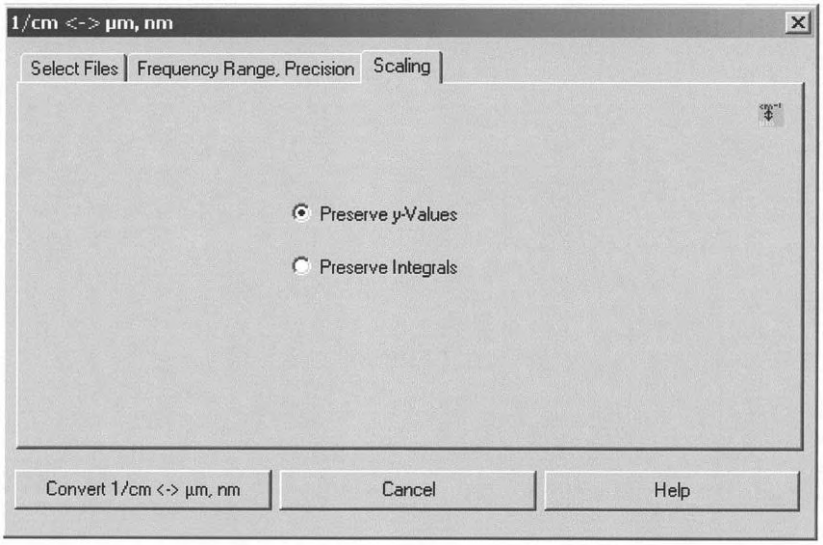


Figure 10.56. The abscissa conversion dialog box: *Scaling* page.

Examples of the two scaling versions are given for the IR spectrum of isopropylmyristate in Fig. 10.57.

10.21 Averaging of Spectra

The *Averaging* function generates an average spectrum from a set of original spectra of the same type.

Select the spectra either by dragging these from the browser on the selection window:
Check *Select by Symbol* see Fig. 10.58a
or by specifying their names:
Check *Select by Name* see Fig. 10.58b.
In the latter case you also have to specify the path and the data block of the files.
If the option *Update Av. Spectrum* is not marked, the calculated average spectrum will be saved as a work file Av.x. If you check the *Update Av. Spectrum* box, an additional field will be displayed in which you have to drag an existing spectrum file to which the average data should be added. Usin the latter version you can carry out the averaging procedure by adding spectra step by step.

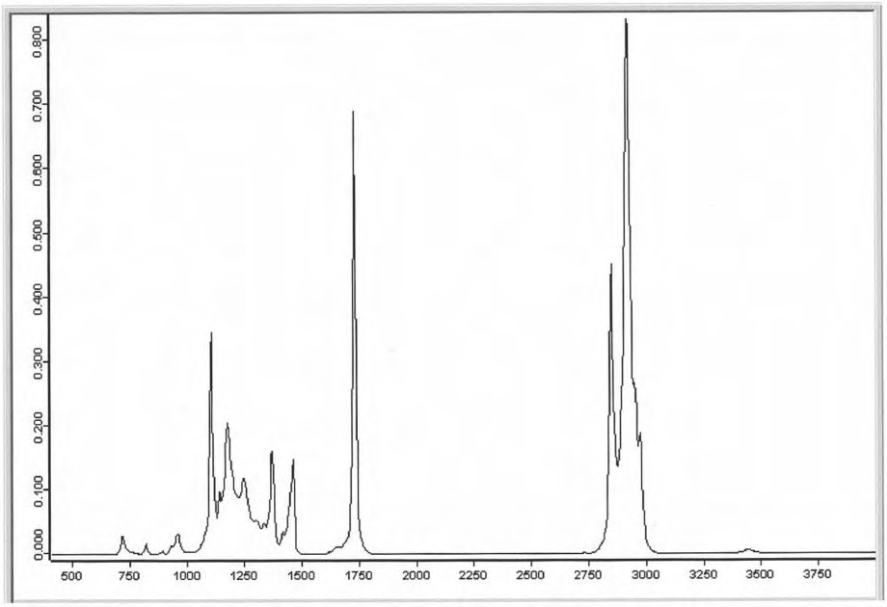


Figure 10.57 a. Legend see next page

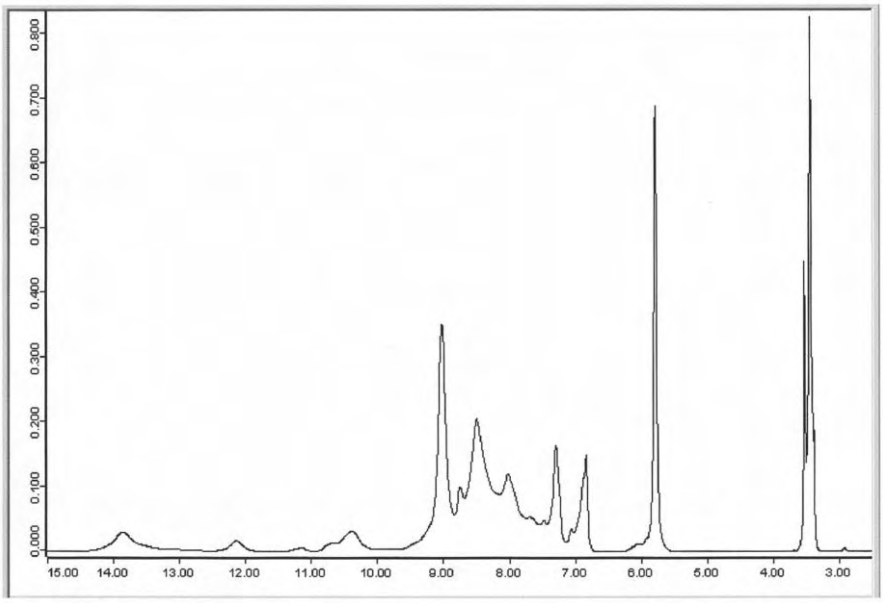


Figure 10.57 b.

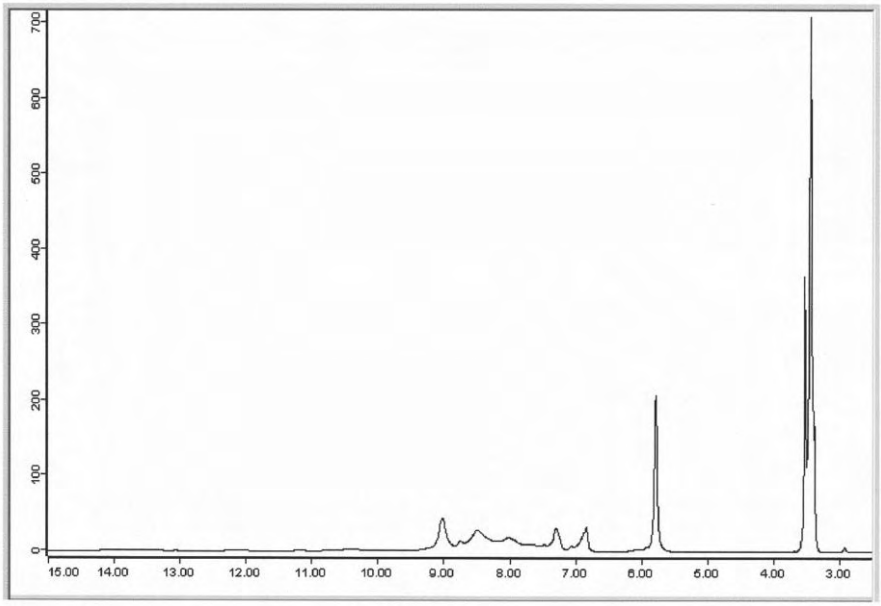


Figure 10.57. c.

Figure 10.57 a–c. a: The IR spectrum of isopropylmyristate scaled in wavenumber units. b: The IR spectrum of isopropylmyristate converted to wavelength units with *Preserve y-Values.* c: The IR spectrum of isopropylmyristate converted to wavelength units with *Preserve Integrals.*

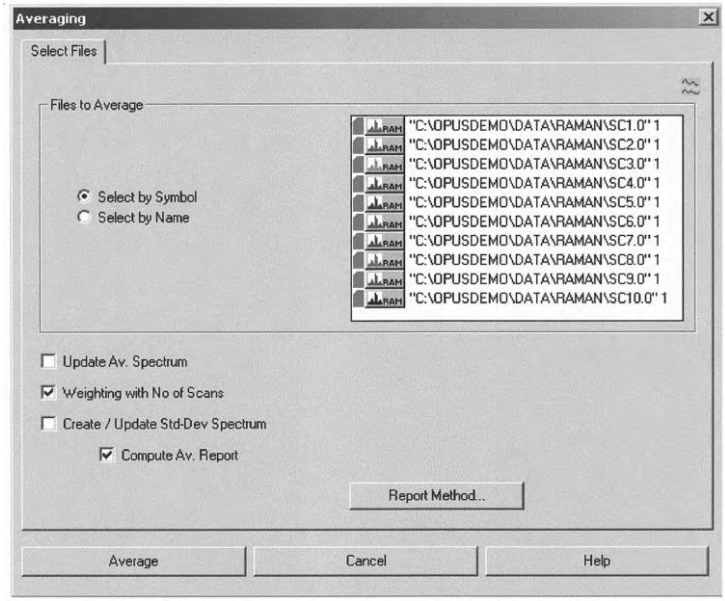


Figure 10.58 a.

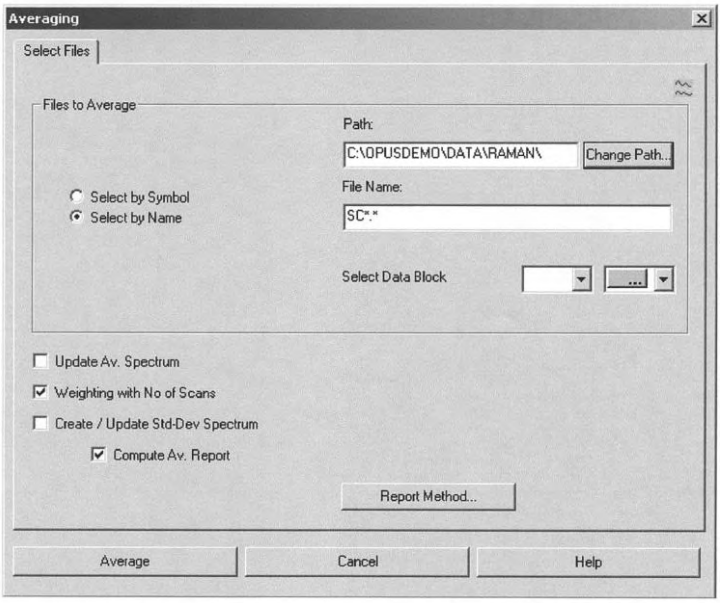


Figure 10.58 b.

Figure 10.58 a The *Averaging* dialog box: files to average are selected by symbols.
b: The *Averaging* dialog box: files to average are selected by names.

If the checkbox *Weighting with No of Scans* is checked, the averaging is carried out by weighting the selected data files. In this case, each spectrum is weighted proportional to the number of scans measured for each file.

If you select *Create/Update Std.-Dev. Spectrum,* a second spectrum is generated, in addition to the averaged spectrum, whose intensity is given by the relative standard deviation of the averaged spectra.

You can also create an average report that contains a comparison of the original set of spectra with the average spectrum. In this report the distances of the averaged spectrum from the original spectra are listed. These distances are given in multiples of the standard deviation in order to make the detection of outliers easier. You will also find listed in the report the method that was used for averaging the spectra and the frequency region of the calculation. Select the method by clicking on the *Report Method* button. The methods are saved in files with the extension .faa.

Upon averaging, the arithmetic mean intensity $\bar{y}$ of the n input spectra is calculated by

$$\bar{y} = \frac{\sum_{i=1}^{n} y_i}{n} \tag{10.25}$$

For n averaged spectra, the standard deviation σ is given by

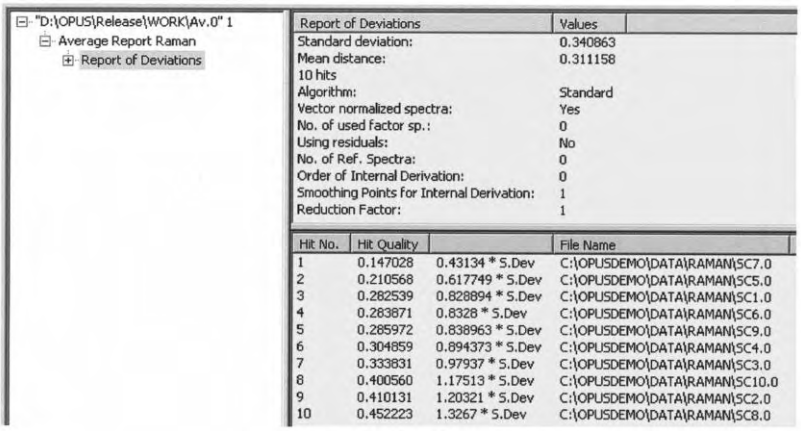
$$\sigma = \sqrt{\frac{\sum_{i=1}^{n} (y_i - \bar{y})^2}{n - 1}} \tag{10.26}$$

Standard deviation spectra calculated with and without weighting the number of scans appear different, even when the number of scans is the same for all spectra. This is due to the equation denominator ($n - 1$ vs. n) where n is the number of scans, not the number of averaged spectra. The result is therefore different by a constant factor. For a large number of input spectra, this factor approaches 1.

To exercise the *Averaging* function, you should consider the ten RAMAN files SC1…SC10 as already shown in Fig. 10.58. These files represent the Raman spectra of stratum corneum or horny layer, the outermost layer of the mammalian skin. The samples studied were taken from the heels of 10 healthy Caucasian volunteers. Compute the averaged spectrum of these samples and the relative standard deviation spectrum. The report of this average is listed in Fig. 10.59.

10.22 Merging Spectra Ranges

Spectra of the same type (e. g. absorbance spectra) can be linked together to form a new spectrum. Any gap in the wavenumber region is filled with a straight line. If two spectra to be merged overlap, linear weighting is per-



"D:\OPUS\Release\WORK\Av.0" 1
Average Report Raman
Report of Deviations

Report of Deviations	Values
Standard deviation:	0.340863
Mean distance:	0.311158
10 hits	
Algorithm:	Standard
Vector normalized spectra:	Yes
No. of used factor sp.:	0
Using residuals:	No
No. of Ref. Spectra:	0
Order of Internal Derivation:	0
Smoothing Points for Internal Derivation:	1
Reduction Factor:	1

Hit No.	Hit Quality		File Name
1	0.147028	0.43134 * S.Dev	C:\OPUSDEMO\DATA\RAMAN\SC7.0
2	0.210568	0.617749 * S.Dev	C:\OPUSDEMO\DATA\RAMAN\SC5.0
3	0.282539	0.828894 * S.Dev	C:\OPUSDEMO\DATA\RAMAN\SC1.0
4	0.283871	0.8328 * S.Dev	C:\OPUSDEMO\DATA\RAMAN\SC6.0
5	0.285972	0.838963 * S.Dev	C:\OPUSDEMO\DATA\RAMAN\SC9.0
6	0.304859	0.894373 * S.Dev	C:\OPUSDEMO\DATA\RAMAN\SC4.0
7	0.333831	0.97937 * S.Dev	C:\OPUSDEMO\DATA\RAMAN\SC3.0
8	0.400560	1.17513 * S.Dev	C:\OPUSDEMO\DATA\RAMAN\SC10.0
9	0.410131	1.20321 * S.Dev	C:\OPUSDEMO\DATA\RAMAN\SC2.0
10	0.452223	1.3267 * S.Dev	C:\OPUSDEMO\DATA\RAMAN\SC8.0

Figure 10.59. The average report for the Raman spectra of 10 stratum corneum samples.

formed to avoid a step. If the total x-range of one of the spectra to be merged covers that of another spectrum, i. e. one spectrum is superfluous, OPUS gives a warning message and stops the function. The parameter set of the new spectrum is copied from the first spectrum to be merged. To merge spectra, drag them on the *Select Files* window of the respective dialog box (Fig. 10.60) and click on the *Merge* button.

To illustrate the merging procedure, consider the Raman spectrum of stratum corneum. There are two spectral regions of interest, namely between 600 and

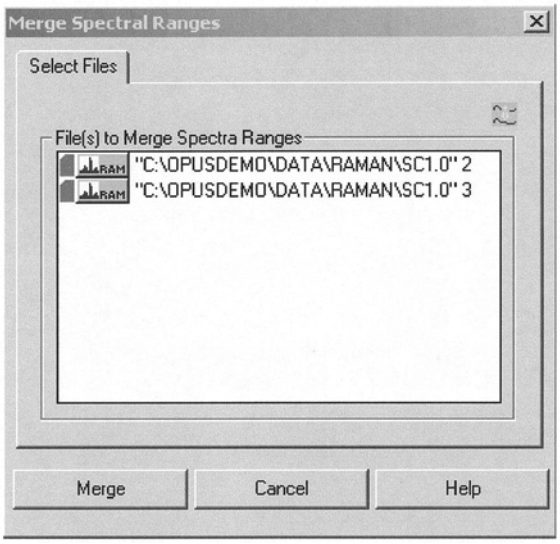


Figure 10.60. The *Merging Spectra Ranges* dialog box.

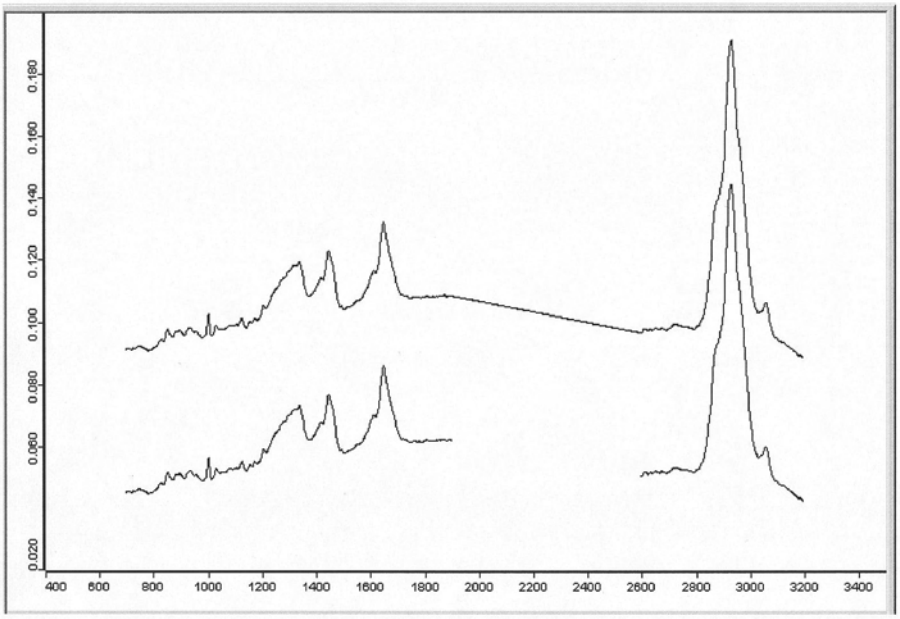


Figure 10.61. Original and merged spectra: bottom, two separated spectra; top, result of merging spectra range, a straight line is inserted between 1900 and 2600 cm^{-1}.

1800 cm^{-1} and between 2700 and 3200 cm^{-1}. Therefore, let us generate two separate spectra by cutting the corresponding ranges and then merge them. The resulting spectrum is shown in Fig. 10.61.

References

1. Savitzky, A., Golay, M. J. E., *Anal. Chem.*, **1964**, *36*, 1627–1639; Correction *Anal. Chem.*, **1972**, *44*, 1906–1909.
2. Bromba, M. U. A., Ziegler, H., *Anal. Chem.*, **1981**, *53*, 1583–1586.
3. Gorry, P. A., *Anal. Chem.*, **1990**, *62*, 570–573.

11 Evaluating

The *Evaluate* menu is primarily intended to derive results from existing spectra. This could be a quantitative data analysis, peak identification or a library search. These functions do not alter the spectrum files. The *Evaluate* pull-down menu is given in Fig. 11.1.

Figure 11.1. The *Evaluate* pull-down menu.

11.1 Curve Fit

Curve Fit is a function used to decompose the area of heavily overlapping bands into constituent components. The implemented procedure is based on the least-squares minimization algorithm. Each band is characterized by the parameters band position, intensity, and width. Furthermore, the type of the band shape is taken into account, whereby you can choose a Gaussian or Lorentzian function or a linear combination of both.

Note that before the fitting calculation is started, it is essential to generate a model consisting of an estimated number of bands and a baseline. The model can be setup interactively on the display and is optimized during the calculation. Since the result of the calculation is highly dependent on the model chosen, care must be taken that the model is reasonable from the chemical point of view. An indispensable part of any fitting procedure is a good choice of initial parameter values.

Calling up *Curve Fit* from the *Evaluate* menu, a dialog box (Fig. 11.2) appears to select the spectrum and the spectral range. On the first page, the spectrum to be fitted is selected by dragging it from the browser window into the *File to Fit* box. Note that the spectrum needs to be of absorbance type and baseline

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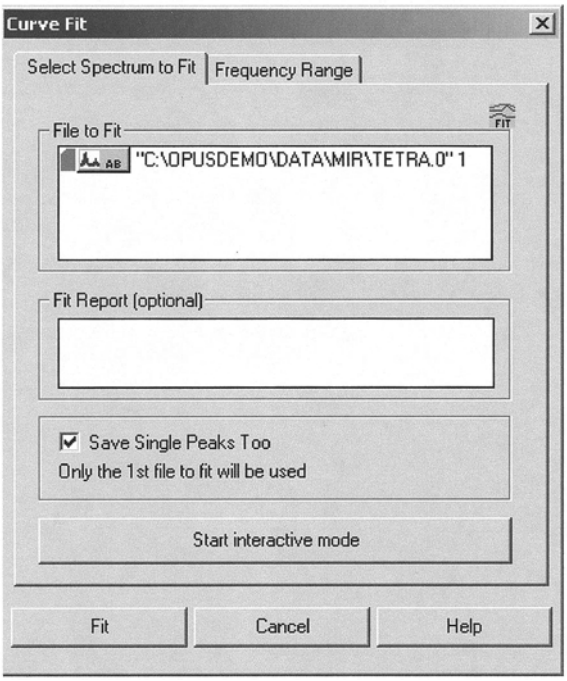


Figure 11.2. The *Curve Fit* dialog box: *Select Spectrum to Fit* page.

corrected. If *Save Single Peaks Too* is checked, every fitted peak will be stored as a separate OPUS file. In the second page of the dialog box, you can select the spectral range of the spectrum you want to fit. Left-click on *Interactive* leads you to a new window where you can decide the area to fit interactively. *Get Display Limits* takes the limits from the respective display window. When ticking *Use File Limit*s, the original range of the file is taken. The previous two options are then blanked out.

In order to perform a curve fit, an appropriate method must first be created. By clicking on the button *Start Interactive Mod*e, the spectrum you selected will be opened in the curve fit setup window (see Fig. 11.3). In the upper window, the spectrum to be fitted is displayed, whereas in the bottom window the difference between the original spectrum and the fitted spectrum is shown. As there was no fitting carried out at this point, both windows show the same spectrum.

You can create a curve fit model by moving the cursor into the top window. The mouse pointer will change from an arrow to ADD. By clicking anywhere in the top window, a curve will appear at the chosen point. Each left-click will generate a new curve. The intensity of the curve corresponds to the cursor position. The generated curves will appear in red. When the cursor is positioned close to the top of the peak, the symbol MOVE is displayed. You can now move the curve by clicking and dragging it to the desired position. To change the width of

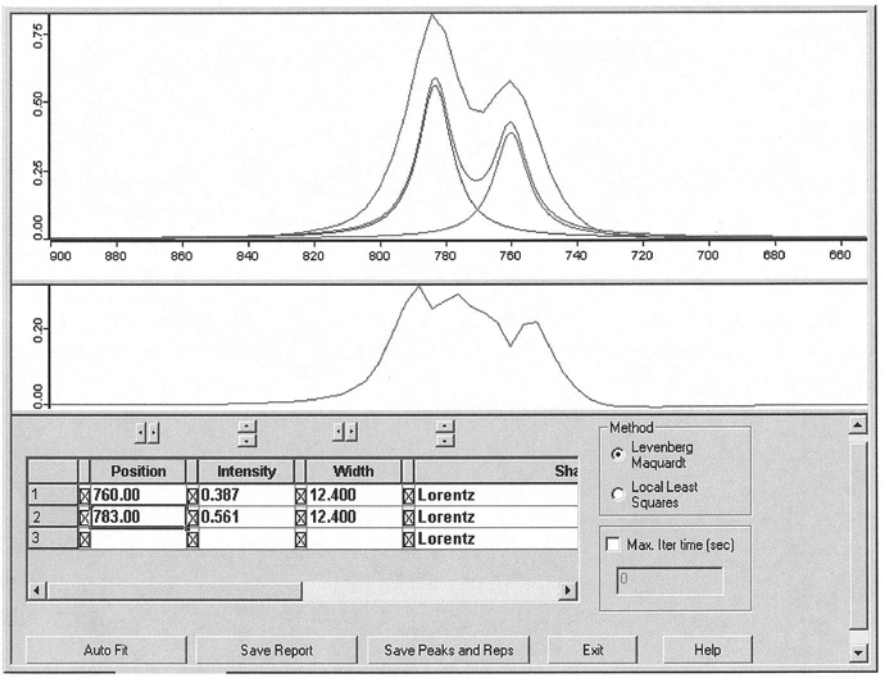


Figure 11.3. The *Curve Fit* setup window before fit. The IR spectrum of carbon tetrachloride (file MIR\TETRA) in the spectral range 660–900 cm^{-1} is displayed together with two Lorentzian functions added.

the peak, position the cursor slightly below the top of the curve and the symbol will appear. By clicking and dragging to the left, the curve will become wider, whereas dragging to the right makes the curve narrower.

11.1.1 Band Parameters

In the bottom half of the window, various parameters can be edited. *Position*, *Intensity* and *Width* can here be manipulated more precisely than with the cursor. To change the parameters of a curve, it must be selected first by clicking on the respective field. Then the values can be directly typed in or changed with the respective arrow key above the field.

11.1.2 Band Shape

The default setting is *Lorentz*, i.e. a pure Lorentzian function. A single click on the upper arrow key switches immediately to a pure Gaussian function. The next click on the same arrow sets the peak to *Baseline*. If, beginning again with the Lorentzian type, the down arrow is clicked on instead, the band shape changes to *100% Lorentz + Gauss*. In principle this band

shape is also a pure Lorentzian function, but it represents the special case of a linear combination of Lorentzian and Gaussian functions. Repeated clicking on the down arrow manually decreases the portion of the Lorentzian function counted as a percentage. We recommend starting the fit process with a fifty:fifty mixture of Lorentzian to Gaussian, because the program determines the best ratio.

11.1.3 Baseline

In some cases, it may be necessary to use a *Baseline* in addition to the peaks. A baseline is always a straight line defined by a reference point and a slope. The baseline can be defined by the operator or calculated by OPUS. In the band list, the parameter *Width* is to read as *Slope*. Baseline parameters can be set interactively with the mouse or directly in the peak table.

The parameters of the baseline are automatically modified only if the *Levenberg–Marquardt* algorithm is used. If the *Local Least Squares* algorithm is selected, the parameters are set manually.

11.1.4 Band List

Each peak is shown in one line of the band list including all parameters. The lines with unselected bands are green and the currently selected band is marked in violet. The bands in the list are always sorted by band position.

11.1.5 Algorithm

Two different algorithms are available for the optimization of the model:

- Levenberg–Marquardt
- Local Least Squares

The algorithm can be selected before starting the calculation, but can also be altered during the calculation. The algorithm principles are described below.

11.1.6 Status Line

The status line below the band list is activated after starting the calculation. It shows the iteration time and the residual RMS error of the fit. The smaller the value of the error the smaller the deviation between measured and calculated curve.

Sometimes an additional message **Bad Fitting Model** is displayed in the status line. This message is displayed if the program was not able to fit one or more bands. These peaks are found at the left or right ends of the frequency range and usually have very small intensities. These bands need to be deleted before the calculation is continued. To delete a band, click on its band number (left row) to select the band and press *Delete* on the keyboard.

11.1.7 Max Iter. Time (s)

The maximum iteration time for *Auto Fit* in seconds is specified in this field.

11.1.8 Auto Fit

The *Auto Fit* button is used to start the calculation. After clicking on this button the calculation begins and the button text changes to *Stop*. During the iteration the newly calculated bands are shown on the display in real time. The calculation can be interrupted at any time by clicking on the button. To restart, click the *Auto Fit* button again.

11.1.9 Save Report

Select the *Save Report* button to save the current report. The resulting sum-spectrum is stored as a temporary work file. This fit report can be used for the curve fit of new spectra.

11.1.10 Save Peaks and Reps

In addition to the previously described functions for the *Save Report* button, the fit report data block FIT.REP is added to the original spectrum.

Once a suitable model has been found it can be applied to other spectra of the same kind; there is no need to setup a new model for each spectrum. The spectra to be analyzed must be selected into the top window.

The fit report block of the already fitted spectrum is then dragged into the second box. Clicking onto *Fit* starts the Curve Fitting of all spectra with the selected method.

11.1.11 Some Examples of Curve Fitting

11.1.11.1 Finding the Shape of a Single Band

Looking for this simplest case, call up the Raman spectrum ETHANOL, select the spectral range between 820 and 940 cm^{-1} and add one curve at 884 cm^{-1} as well as a baseline at 830 cm^{-1}. Further, check *Levenberg–Maquardt* and *Max. Iter time (s)*, mark all parameters and finally click on *Auto Fit.* The result of the fitting should be: band position 883.117 cm^{-1}; intensity 0.120; width 14.239 cm^{-1}; 67% Lorentzian + 34% Gaussian; and residual RMS 0.000305. If your first attempt does not provide these parameter values, start the process again entering changed values to improve the fit. You can see that curve fitting is a kind of trial and error.

11.1.11.2 Decomposition of Two Overlapping Bands

Consider the MIR spectrum of carbon tetrachloride (MIR\TETRA) that exhibits a spectral feature of two overlapping bands at about 780 cm^{-1}. Choose the suitable spectral range, check *Save Single Peaks Too,* start the interactive mode and enter two curves initially. Then the curve fit set-up window could look like that shown in Fig. 11.3. For better initial parameter values add a baseline and alter the shape of both curves to a fifty : fifty mixture of Lorentzian and Gaussian functions. Follow the same steps as mentioned above and you will find that the fitting procedure converges after a very short time. The result is shown in Fig. 11.4. By clicking on the button *Save Peaks and Reps* you can save the fit report and every fitted peak will be stored as a separate file named FIT.

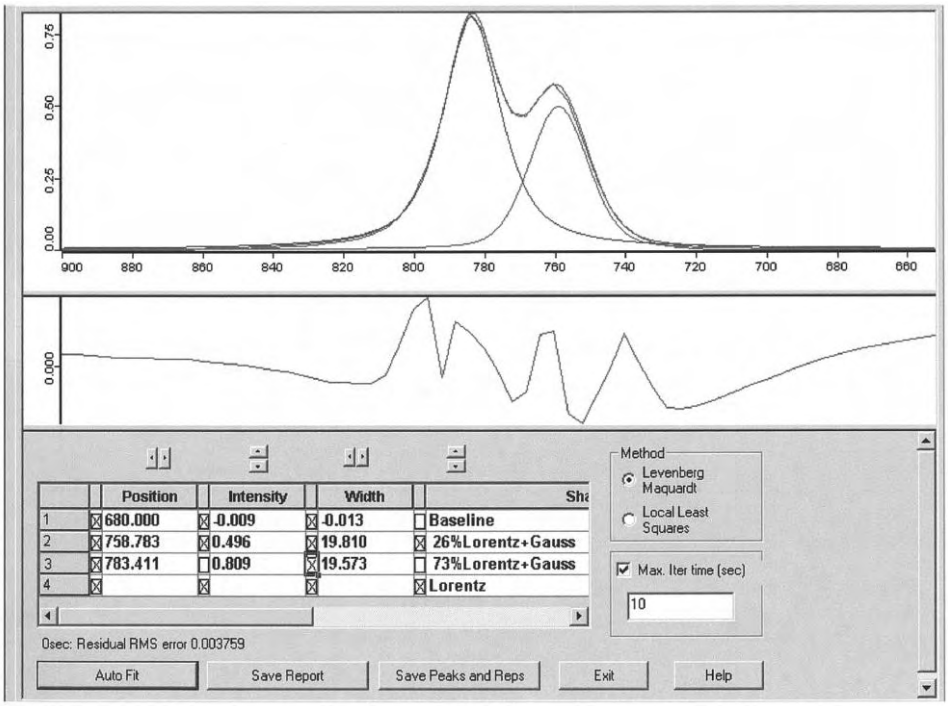


Figure 11.4. The *Curve Fit* setup window after fit, showing the result for the overlapping IR bands of carbon tetrachloride.

The fit report is given in Fig. 11.5. Of course all fitted bands can be also represented as spectra, as illustrated in Fig. 11.6. In this way it is possible to visually compare the original spectrum with the theoretical spectrum consisting of all constituent bands and the baseline.

Curve fit parameters	Values
C:\OPUSDEMO\DATA\MIR\TETRA.0	
Number of peaks:	3.000000
Algorithm: Levenberg-Marquardt	
Max. Iteration time:	10.000000
x-Start:	900.000000
x-End:	650.000000
Residual RMS error:	0.003759

	Position		Intensity		Width	Integral		Shape
	680.000000		-0.009132		-0.013362	0.000000	*	Baseline
	758.782623		0.495664		19.810404	11.758228		26%Lorentz+Gauss
	783.410583	*	0.809191		19.572569	22.712994	*	73%Lorentz+Gauss

Figure 11.5. The *Curve Fit* report for the IR bands of carbon tetrachloride.

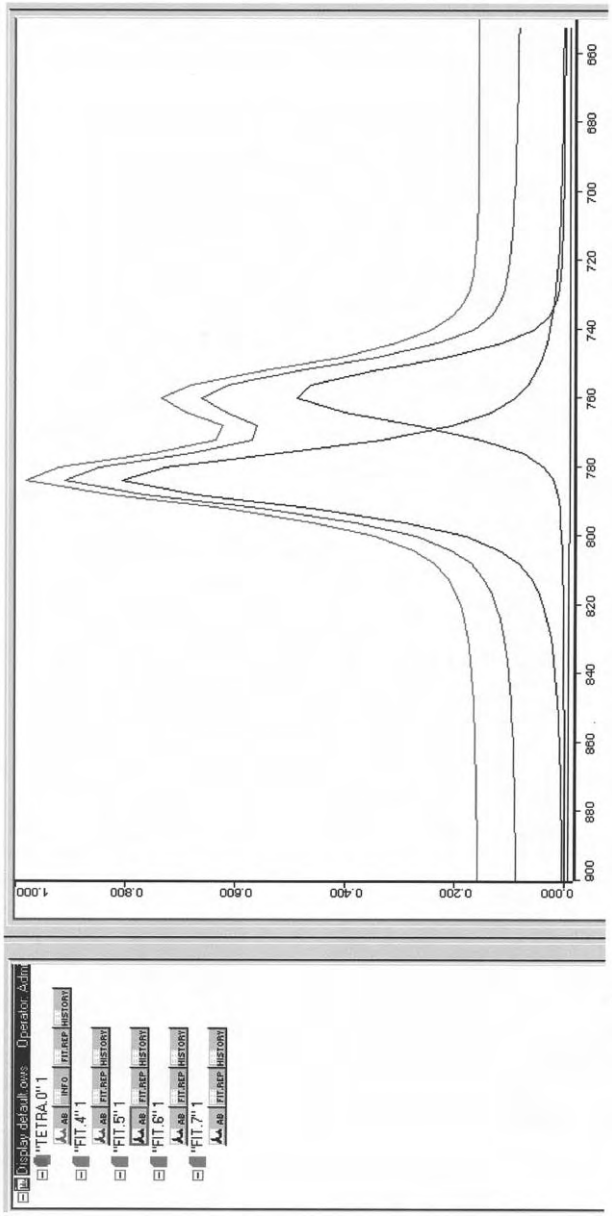


Figure 11.6. Comparison of the original and fitted IR spectra of carbon tetrachloride. For clarity the curves are shifted: from top to bottom, original spectrum, fitted spectrum, two fitted bands, and baseline.

11.1.11.3 Decomposition of a Complex Spectral Feature

Call up the Raman spectrum of n-docosane (RAMAN\ALKAN22), which exhibits a rather complex spectral feature in the C–H stretching region between 2800 and 3000 cm^{-1}. The fit result of this band based on seven curves is shown in Fig. 11.7. Verify this decomposition We also propose to look for the temperature-induced changes of the fitted bands using the Raman spectra RAMAN\ALKANC22T.n. Puzzle out which bands show strong temperature dependence.

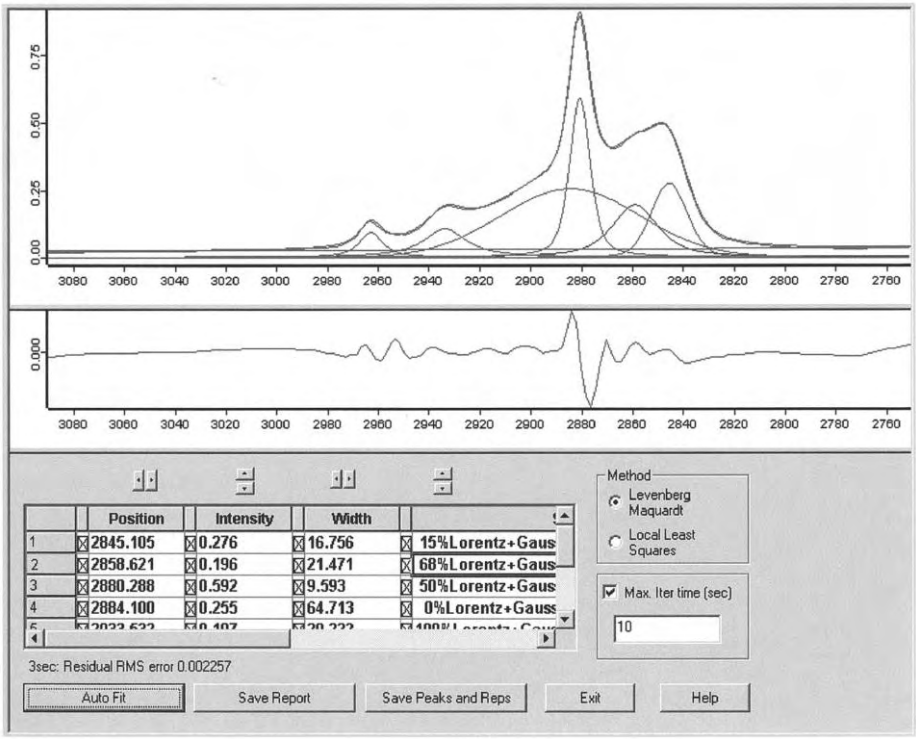


Figure 11.7. The *Curve Fit* setup window after fit, displaying the result for the Raman spectrum of n-docosane (file RAMAN\ALKAN22) in the C–H stretching region between 2760 and 3080 cm^{-1}.

11.1.12 Theoretical Background

Two algorithms are implemented in OPUS:

- *Levenberg–Marquardt*
- *Local Least Squares*

Both algorithms are based on the least squares method. The difference between the original curve $Y(\tilde{\nu}_i)$ and the calculated curve $y(\tilde{\nu}_i,S)$ is kept as small as possible. Here S stands for the set of band parameters

In the frame of the *Levenberg–Marquardt* algorithm the sum of the quadratic deviations between measured and calculated data points is given by

$$\chi^2(S) = \sum_{i=1}^{N} \left[Y(\tilde{\nu}_i) - y(\tilde{\nu}_i;S)\right]^2 \tag{11.1}$$

where N is the number of data points. This function is minimized by iteration. Based on the current set of band parameters the gradient of the function 11.1 is calculated. The gradient is then used to determine a new set of parameters S. Some additional restrictions have also been implemented to make the calculation more effective, namely:

- All bands must lie within the specified spectral range.
- The width of a band must not be greater than the specified spectral range.
- Band intensities must be positive.
- The contribution of Lorentzian and Gaussian functions for mixtures must lie within the range 0% to 100%.
- The sum of both parts must always be 100%.

The *Local Least Squares* algorithm performs an independent fit for each individual peak. The calculation is thereby restricted to the range around the band maximum. This drastically reduces the amount of data required for the calculation, enhancing the speed compared to the *Levenberg–Marquardt* method. Some loss of precision versus the *Levenberg–Marquardt* method occurs. The *Local Least Squares* algorithm has some conditions:

- The parameters for the baseline are taken from the model, not calculated.
- The band parameters are always variables; none of them can be fixed.

11.1.12.1 General Procedure

The given model can be considered as an area in an n-dimensional space with n being the total number of band parameters. In most cases this area has one absolute minimum and several local minima. The quality of the calculation depends on the quality of the selected model, i.e. does the calculation find the absolute minimum or does it orbit in the vicinity of the local minima. The latter case can be detected by a relatively large error and a visually obvious poor fit. In the case of a poor fit, start again with a new optimized model.

11.1.12.2 Criteria for the Selection of an Algorithm

In most cases the *Levenberg–Marquardt* algorithm gives a better fit compared to the *Local Least Squares* algorithm, but needs significantly more calculation time. If the number of points in the selected region and the number of bands in the model are small, the *Levenberg–Marquardt* algorithm can be used immediately. If the amount of data is large or many bands need to be fitted, start with the *Local Least Squares* algorithm that converges very quickly. As soon as the variation of the error becomes small, switch to the *Levenberg–Marquardt* algorithm for the final fit.

As with any iterative procedure, the biggest problem is that you do not even know if the routine has reached an absolute or relative minimum. The only way that you can "make" the iterative procedure get to an absolute minimum is to start fitting from several different initial parameter values and see what happens. If you always obtain the same final results, the result is less likely to represent a local minimum.

11.2 Integration

This function is used to carry out the integration of bands as well as the calculation of peak heights. As usual load the spectrum of interest on the *Select Files* page of the *Integration* dialog box (Fig. 11.8). Of course the user must specify the integration method, i.e. define both the wavenumber ranges and the type of integration. OPUS offers 17 different integration types. When you want to create a new integration method, click on the *Setup Method* button and you have access to the *Setup Integration Method* dialog box (Fig. 11.9a). In this box you have to specify the *Left* and the *Right Edge* of the *integration* area of interest and to assign each peak an integration type. Depending on the integration type you should also define two or four baseline points (see Fig. 11.9b).

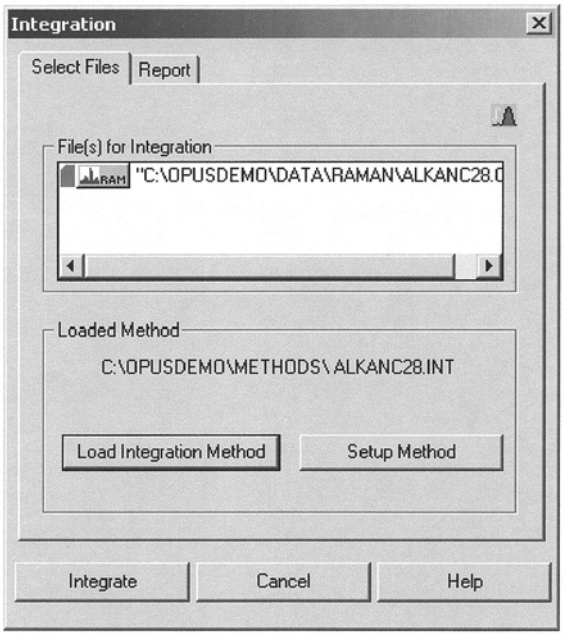
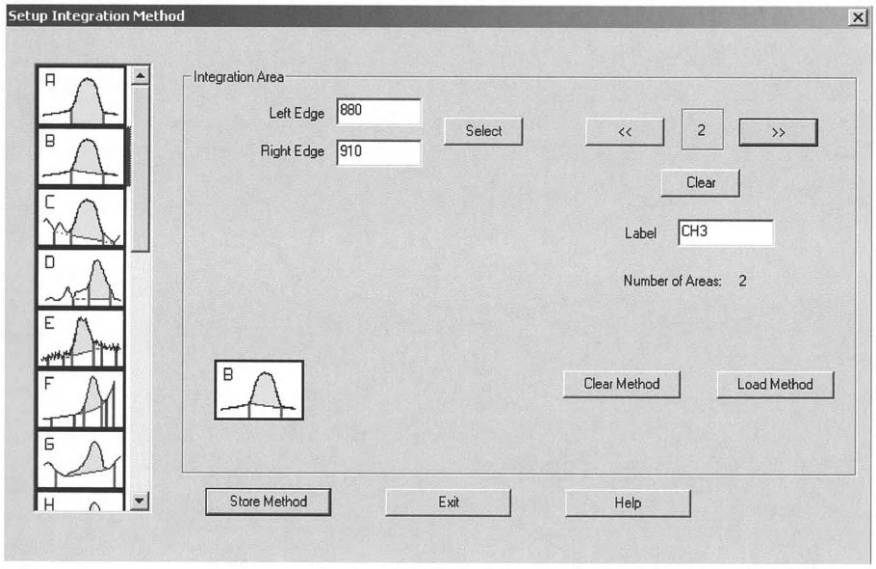
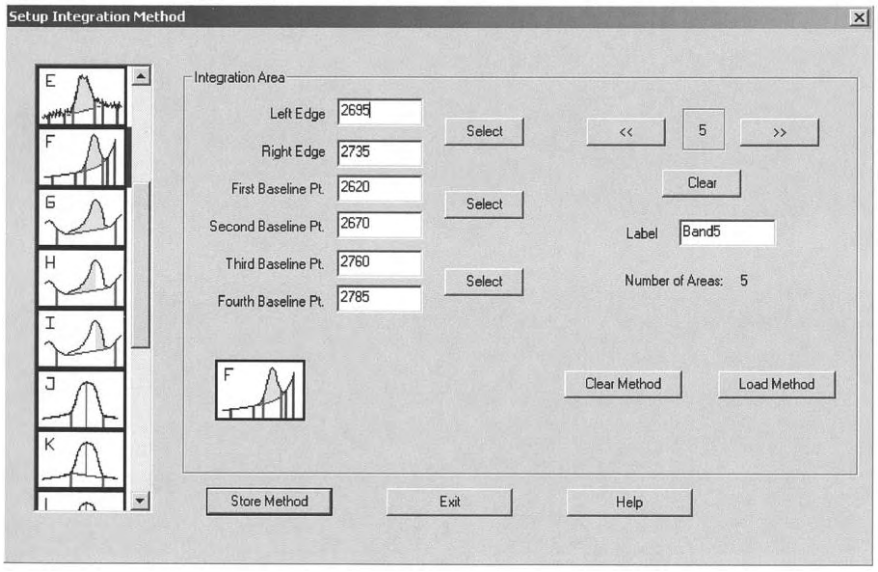


Figure 11.8. The *Integration* dialog box: *Select Files* page.



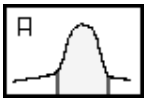
a



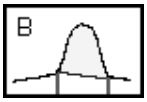
b

Figure 11.9. The *Setup Integration Method* dialog box: Integration area defined by (a) two parameters and (b) six parameters.

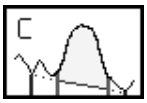
By clicking on the appropriate icons in the left panel you can activate the desired kind of integration. The meaning of the icons is as follows:



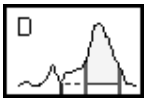
The integration will be performed between the wavenumber limits and the peak envelope, using zero as the baseline.



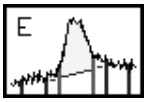
The integration will be performed using as a baseline a straight line that connects the wavenumber limits and the peak envelope.



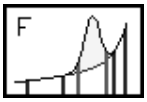
Same procedure as in B, but two separate data points will be used to define the baseline.



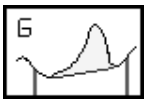
Same procedure as in B, but a straight line running through an additional point will be used as baseline.



Same procedure as in B, but the two points defining the baseline are averaged from the first and the second, the third and the fourth point, respectively. The average values together with the second and third point define the baseline. This is useful for “noisy” spectra.

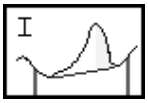


Same procedure as in B, but the baseline will be defined by a least square fit of a parabola to the data points between the first and the second, and between the third and the fourth baseline point.

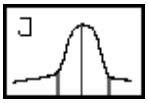


Looks for the highest intensity in the wavenumber range and for the minima left and right from this maximum. The baseline in this region is then defined by the local minima.

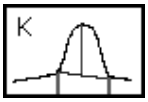
Integrates from the maximum to the left minimum.



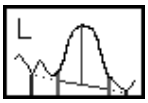
Integrates from the maximum to the right minimum.



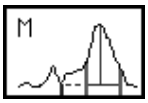
Highest absolute intensity of the peak.



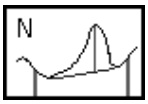
Peak intensity relative to the local baseline.



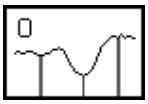
Peak intensity relative to the baseline as defined in C.



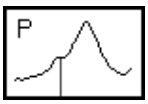
Peak intensity relative to the baseline as defined in D.



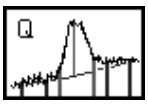
Peak intensity relative to the baseline as defined in F.



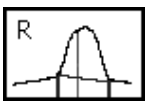
Minimum intensity between the wavenumber limits.



Intensity at the specified wavenumber.



Peak intensity relative to the baseline as defined in E.



Intensity at the specified wavenumber relative to the baseline as defined in B.

The selected integration type for the chosen integral area is displayed to the right of the icon list. Each integration area must be assigned to a label.

Integration methods can be stored separately (*Store Method* button) and loaded at any time (*Load Method* button). The last integration method used becomes the default method until another method is loaded or a new method is created.

The currently loaded integration method is indicated on the first page of the dialog box; this is, in Fig. 11.8, for instance, the method “ALKANC28.INT”.

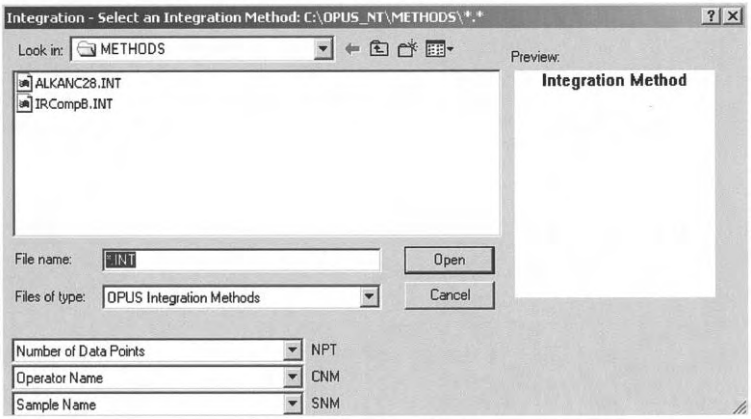


Figure 11.10. The *Select an Integration Method* dialog box.

If no integration method is available, this text reads *"No Integration Method Loaded"*. In this case an existing method must be loaded or a new method must be created.

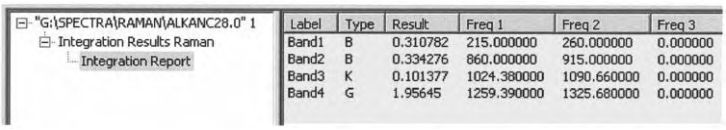
In the former case, click on the *Load Integration Method* button and the *Select an Integration Method* dialog box (Fig. 11.10) appears. Choose the appropriate directory path, select the desired integration method and load that one by clicking on the *Open* button.

Performing the integration generates an integration report that is represented in the browser by the data block icon INTEG. The report contains, in addition to the numerical results of the integration, the method used and the wavenumber limits. An example of such a report is displayed in Fig. 11.11.

If the same spectrum is integrated a second time, you can choose between overwriting the first integration report, merging both reports, or appending the new report into the old one. The default setting will overwrite the report.

For this purpose open the *Report* page (see Fig. 11.12) and mark the relevant item:

- *Overwrite* – replaces the old report (default setting),
- *Merge* – adds the results to an existing report,
- *Append* – generates a new report and adds it to the existing file.



Label	Type	Result	Freq 1	Freq 2	Freq 3
Band1	B	0.310782	215.000000	260.000000	0.000000
Band2	B	0.334276	860.000000	915.000000	0.000000
Band3	K	0.101377	1024.380000	1090.660000	0.000000
Band4	G	1.95645	1259.390000	1325.680000	0.000000

Figure 11.11. An example of an integration report.

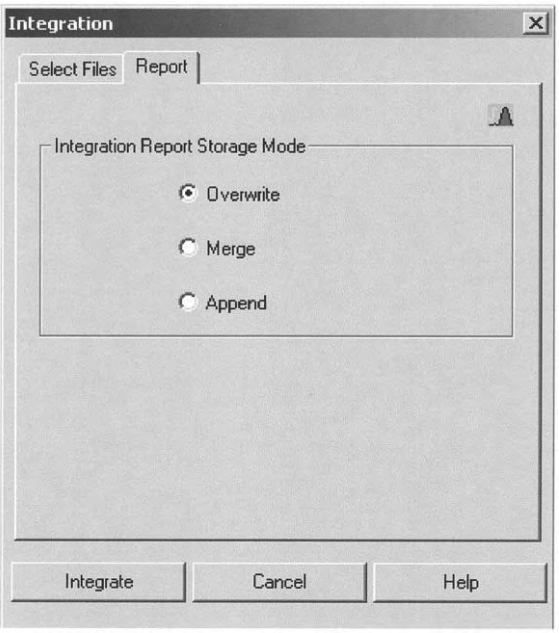


Figure 11.12. The *Integration* dialog box: *Report* page.

11.3 Peak Identification

The *Peak Picking* command helps you to identify peaks in your spectra. The command offers high flexibility by allowing you to adjust and fine tune the search parameters.

The respective dialog box comprises four pages as shown in Fig. 11.13. First, select the spectrum and the spectral region of interest as usual. Then define the *Sensitivity* on the file selection page. Note that the *Sensitivity* is the most important parameter for the identification.

Now you can either choose the interactive mode or switch to the *Mode* page to continue with the automatic mode.

Click on the *Start interactive mode* button to switch to the interactive mode. According to the threshold value set on the slider the *Sensitivity* and with that the number of identified peaks changes. Two examples for this interactive procedure are shown in Fig. 11.14 a and b. Click on the *Store* button to save the results of peak picking.

If you want to apply the automatic mode, call up the *Y-limits* page (Fig. 11.15). Depending on your data, i.e. whether low-intensity peaks are crucial or not, here you can specify the intensity range important to your problem. In this manner it is possible to reduce the hit number. If you mark *Peak < [%]* you can define the peak intensity in relation to the surroundings of the peak and the baseline.

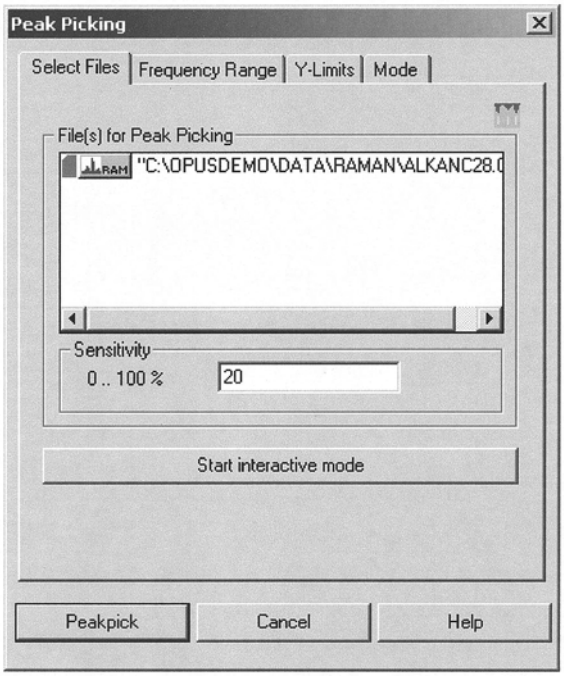


Figure 11.13. The *Peak Picking* dialog box: *Select Files* page.

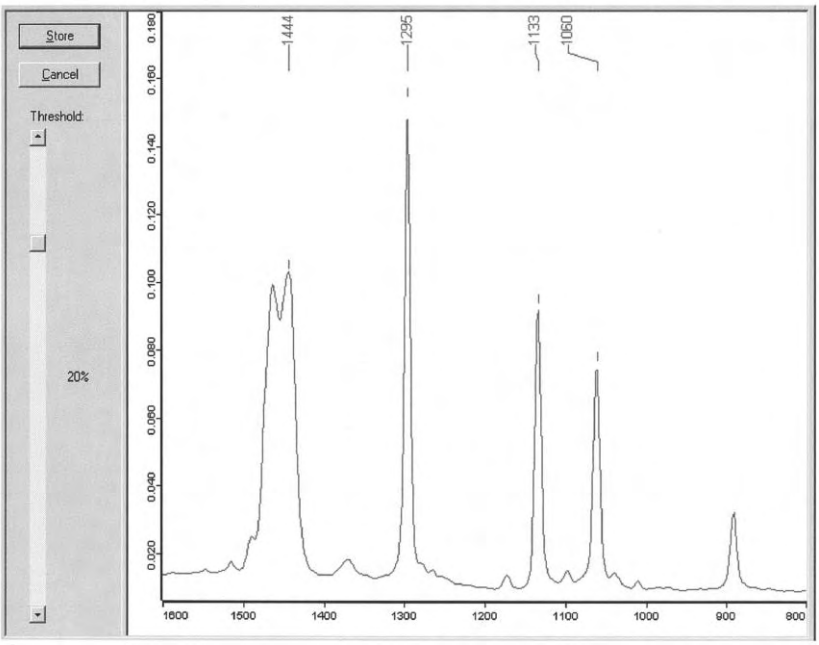
Checking *Peak < [abs] and Peak >[abs]* you can also specify the limits as absolute absorption values. An example of this procedure is shown in Fig. 11.16. Note if you have chosen the interactive mode the parameters marked on the *Y-limits* page are not taken into account.

In the case of absorbance or Raman spectra usually the maxima are of interest, whereas for transmittance or reflectance spectra the minima are the important features. This can be accounted for by selecting the appropriate peak picking mode on the *Mode* page (Fig.11.17). If you mark *Automatic* in the *Direction of Peaks* box, the spectrum type information stored in the data block is used to determine whether maxima or minima are picked. In rare situations, e.g. for subtraction, it is necessary to specify whether the algorithm should pick for minima or for maxima.

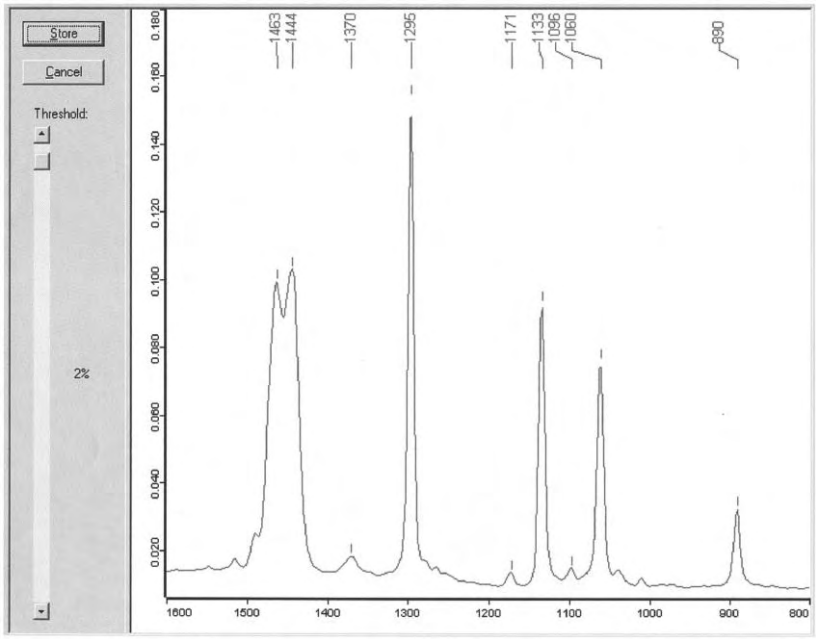
On the *Mode* page you also have to decide the algorithm for peak picking.

The *Standard* method is used for peaks with small or no overlap in spectra of low or average spectral resolution (0.5 cm^{-1} or above): the wavenumber of the band of interest is the x-value of the interpolated maximum or minimum and the peak intensity is the y-value at this point.

The *Second Derivative* method is typically applied in order to be independent of the baseline. As in the case of the *Derivative* function (see Section 10.11) the Savitzky–Golay algorithm is actually used to obtain the derivative. Again, the number of smoothing points used can be adjusted to suppress the effect of



a



b

Figure 11.14. The *Interactive Mode* of peak picking for the Raman spectrum of n-docosane in the spectral range 800–1600 cm^{-1}: (a) threshold value of 20% and (b) threshold value of 2%.

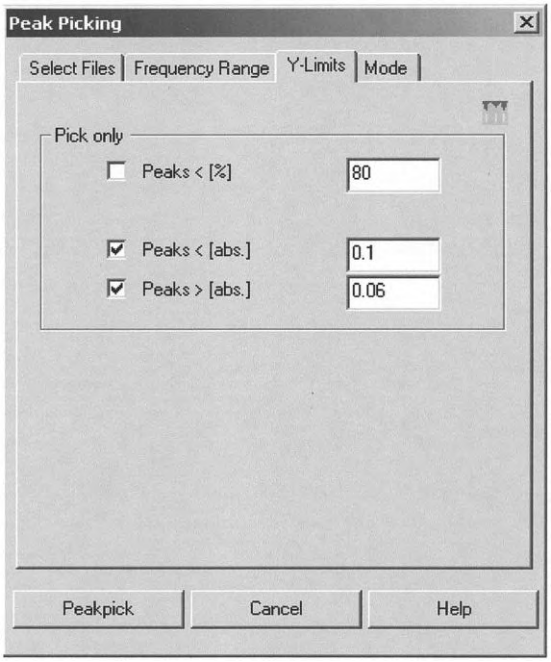


Figure 11.15. The *Peak Picking* dialog box: *Y-Limits* page.

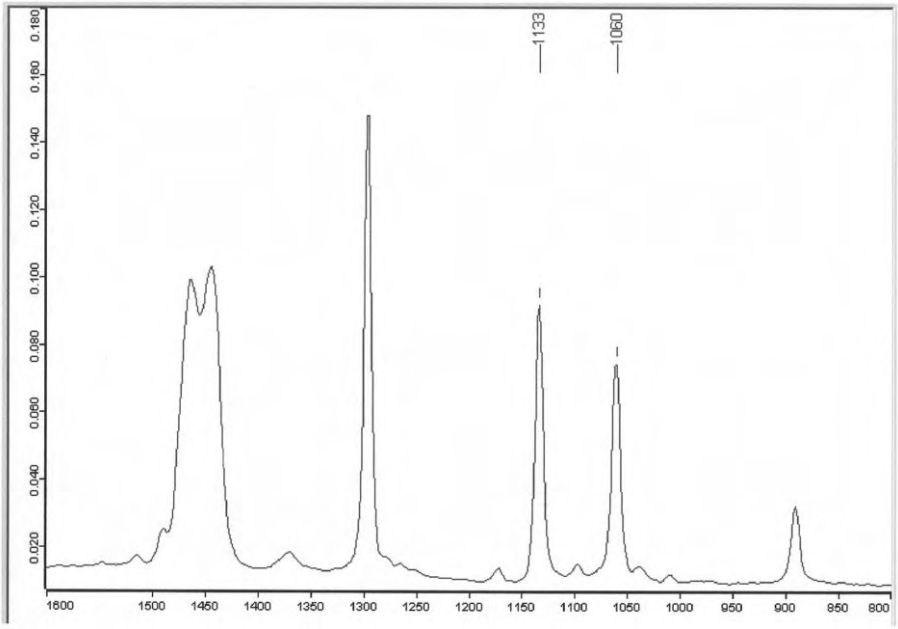


Figure 11.16. Peak picking for the Raman spectrum of n-docosane in the spectral range 800–1600 cm^{-1} using the parameter values *Peak < [abs] 0.1* and *Peak > [abs] 0.06.*

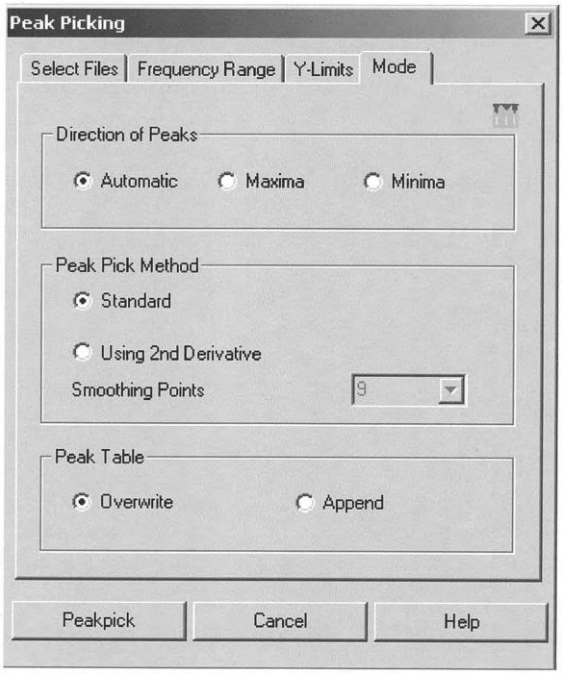


Figure 11.17. The *Peak Picking* dialog box: *Mode* page.

noise. As a rule of thumb, one should not use a larger number of smoothing points than the FWHH of the smallest band of interest. The example depicted in Fig. 11.18 illustrates that the x-values of the minima of the smoothed second derivative spectrum are the positions of the absorption bands of interest. This is particularly useful for seriously overlapping bands because in this case an extremity of the spectrum is frequently shifted with respect to the true band position. Weak shoulders can also be recognized using the second derivative method. Peak picking using the second derivative method is recommended for high-resolution spectra only.

If you use *Peak Pick* on a spectrum that already has a peak list associated, you can choose on the *Mode* page whether to *Overwrite* the old list with the new one or to *Append* the new list to the existing one.

Performing peak identification generates a peak pick report that is represented in the Browser by the data block icon PEAKS. To access the peak table, right-click on the peak pick report block and select the *Show Peak List* command from the pop-up menu. Two examples of such peak reports are listed in Fig. 11.19 and 11.20, namely those for the Raman spectrum ALKANC22 in the spectral range from 800 to 1600 cm^{-1} using the interactive mode with a sensitivity of 2% and the automatic mode with the parameter values *Peaks > [abs] 0.06 ; Peaks < [abs] 0.10*, respectively. As you can see, the essential parameters listed in the report are the wavenumber and the absolute intensity (peak height). The

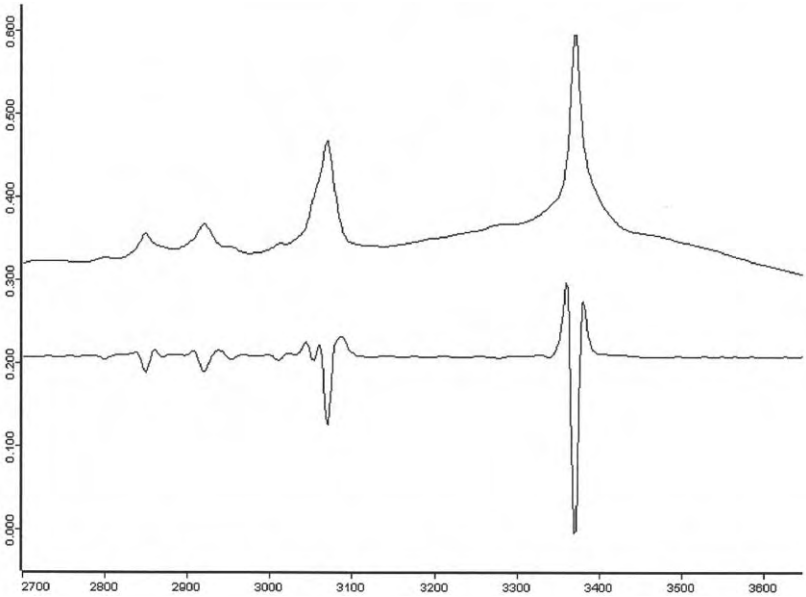


Figure 11.18. IR spectrum of indigo. Top: absorbance spectrum; bottom: second derivative.

"G:\SPECTRA\RAMAN\ALKANC22.0
Peak Table Raman
Peak Picking

Peak Picking	Values
Method:	Standard
Searched for minima:	No
Number of peaks:	9
Sensitivity > [%]:	2.000000
From:	1600.000000
to:	800.000000
Peaks > [abs.]:	0.000000
Peaks < [%]:	0.000000
Peaks < [abs.]:	0.000000

Wavenumber	Abs. Intensity	Rel. Intensity	Width	Found if Threshold <	Shoulder
1463.3	0.100	0.012	7.6	7.879970	0
1443.6	0.104	0.091	12.0	65.072296	0
1370.0	0.019	0.005	18.5	3.446338	0
1295.4	0.154	0.147	7.8	102.235275	0
1171.2	0.014	0.004	9.5	2.927660	0
1133.1	0.094	0.084	8.4	60.066711	0
1096.3	0.015	0.003	8.2	2.344009	0
1060.1	0.076	0.065	8.8	46.404610	0
889.7	0.033	0.024	9.4	16.748158	0

Figure 11.19. Peak pick report for the Raman spectrum of n-docosane in the spectral range 800–1600 cm^{-1} based on the interactive mode with a sensitivity of 2%.

relative intensity also given is of interest for a particular purpose, which is beyond the scope of this book.

By default OPUS determines the number of decimal places used for the wavenumber value from the spectral resolution. On the other hand, the user has the option to define this number in the user settings menu: see *Setup* → *User Settings* → *Preferences*.

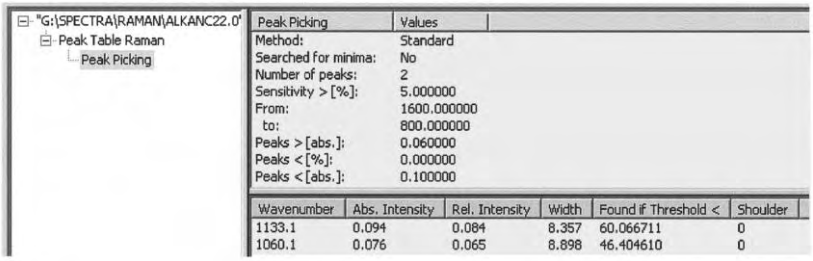


Figure 11.20. Peak pick report for the Raman spectrum of n-docosane in the spectral range 800–1600 cm^{-1} based on the automatic mode with the parameter values *Peak < [abs] 0.1* and *Peak > [abs] 0.06.*

11.4 Quick Identity Test

The *Quick Identity Test offers* a user-friendly method to judge the similarity of two spectra. This method requires a reference spectrum of the substance of interest. The test determines the Euclidean distance between the test and the reference spectra.

Enter the reference spectrum in the field *Principal File for Quick Identity Test,* and the files to be tested in the field *File(s) for Quick Ident* of the *Selected Files* page of the dialog box (Fig. 11.21).

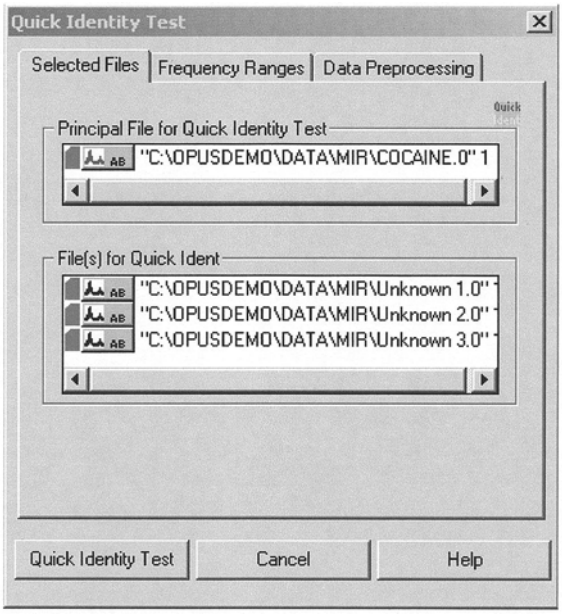


Figure 11.21. The *Quick Identity Test* dialog box: *Selected Files* page.

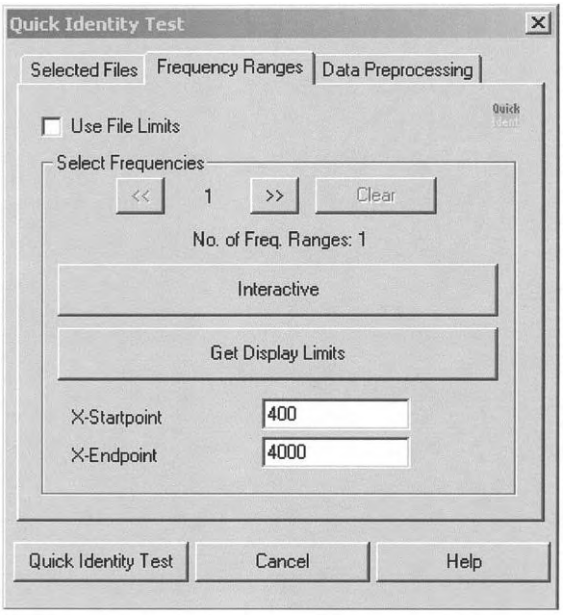


Figure 11.22. The *Quick Identity Test* dialog box: *Wavenumber Ranges* page.

You can define several spectral regions on the second page (Fig. 11.22); only the data of the regions indicated here will be compared. Or you can compare the spectral range common to all spectra by checking the *Use File Limits* box. Switch between regions with the arrow key. Delete a region by clicking on the *Clear* button.

On the *Data Preprocessing* page (Fig. 11.23) select one of the two data preprocessing methods: either the derivative or the vector normalization. The vector normalization causes the Euclidean distance to fall within an interval of 0 and 2.

A report data block REPORT will be appended to every spectrum that was compared to the reference spectrum. This *Report of Correlation Search* (see Fig. 11.24) lists the "Hit Quality", the sample name (as taken from the spectrum parameter block), and the file name. The hit quality should preferably be a small number, with zero being an absolute match. The exemplary report listed in Fig. 11.24 proves that the unknown sample 2 is cocaine; the hit quality is 0.000000.

In addition, a new file "QIdnt.0" will be created, which summarizes the reports of all spectra tested (see Fig. 11.25).

The following information is of relevance when you apply the Quick Identity Test:

Method file:	file name of the method used for the test.
Expected Reference:	the principal file used.

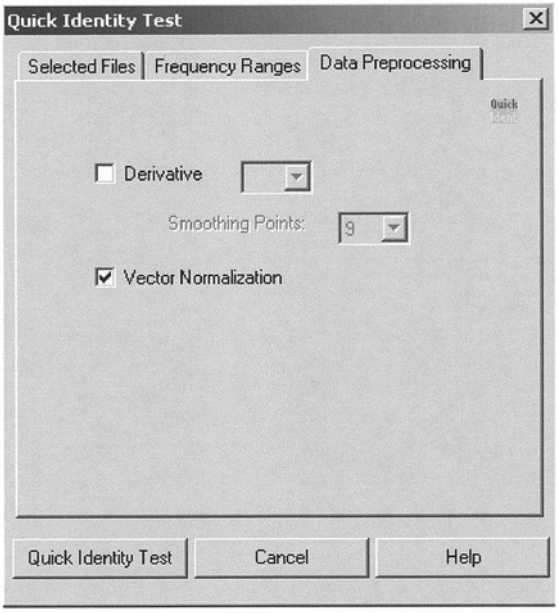


Figure 11.23. The *Quick Identity Test* dialog box: *Data Preprocessing* page.

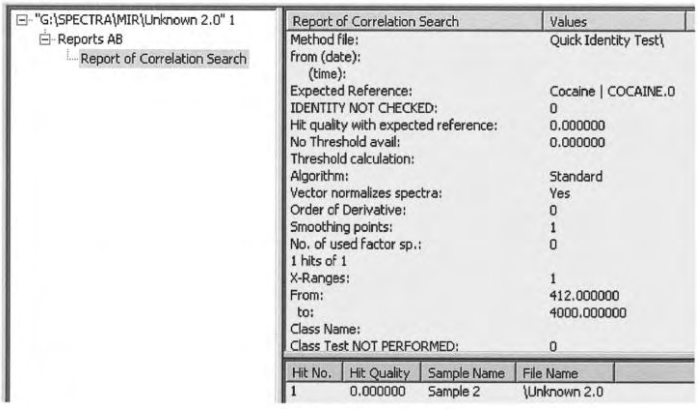


Figure 11.24. An example of a quick identity test report.

Vector normalized spectra:	indicates that vector normalization was used.
X-Ranges:	number of wavenumber ranges.
Order of Internal Derivation:	gives the order of the derivative used.
Smoothing Points for Internal Derivation:	gives the number of smoothing points.

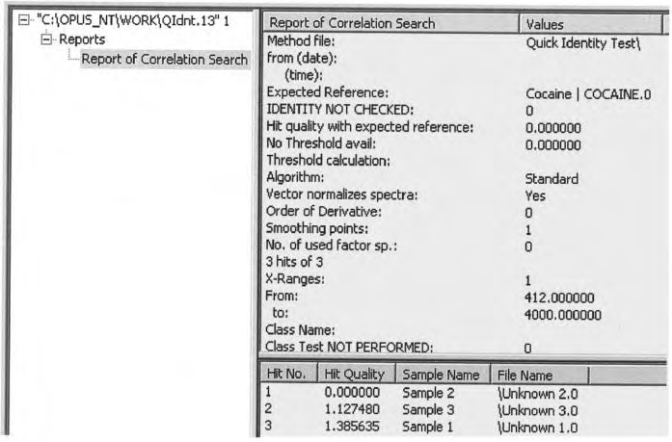


Figure 11.25. A new file "Qidnt.0" summarizes the report of all spectra tested.

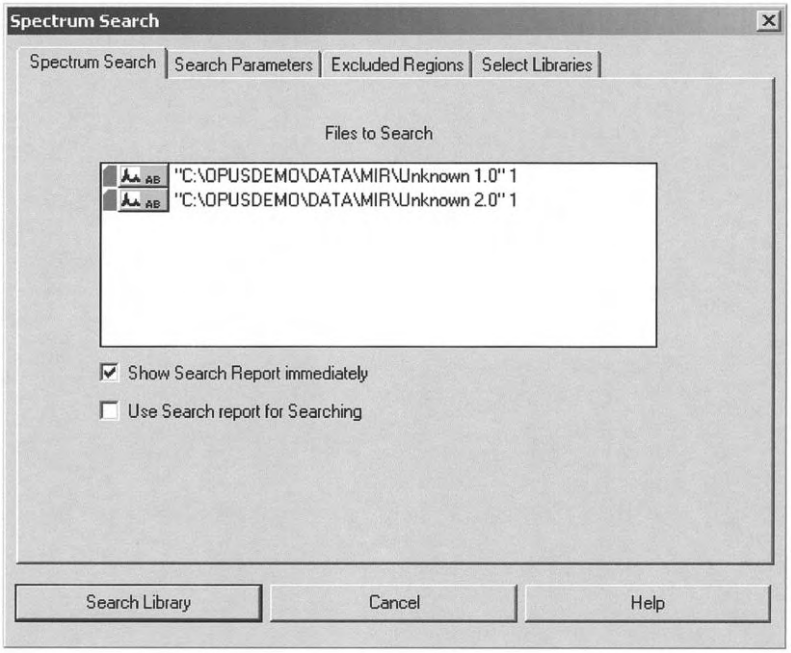
11.5 Spectrum Search

The goal of the spectrum search is the identification of unknown substances based on a reference spectra database. In other words, to perform a search you need a library suited to your problem. The OPUS demo version offers two libraries containing 350 IR spectra and 246 Raman spectra, respectively.

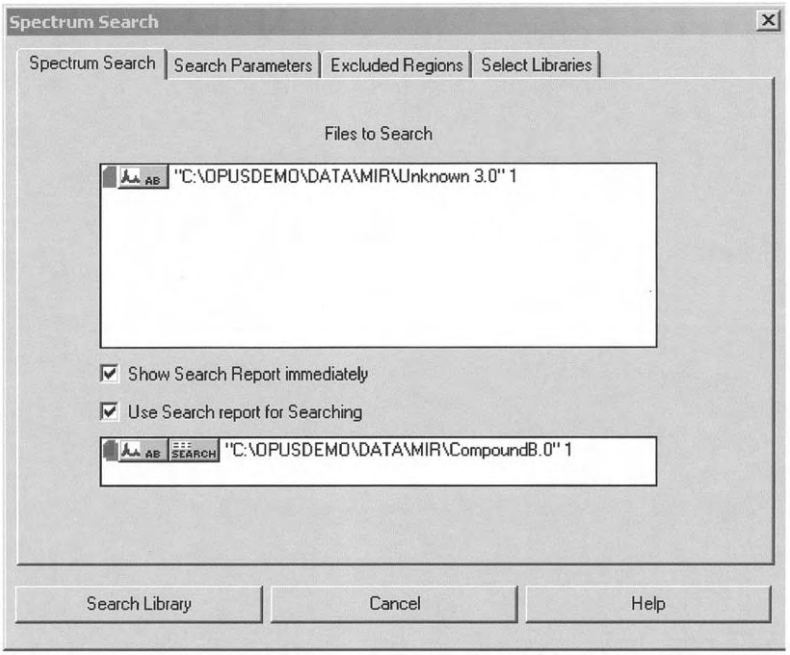
On the first page of the *Spectrum Search* dialog box (Fig.11.26a) you have again to specify the spectrum files for the search run. If the *Show Search Report immediately* checkbox is selected, a window displaying the results opens automatically after the search run. You can limit the search also to an existing search report (see Fig. 11.26b). In this case only the substances listed in the report will be compared to the test spectrum.

On the *Search Parameter* page (Fig. 11.27) you define the desired search parameters:

- *Algorithm*: OPUS search algorithm uses a peak table together with the spectral data. A temporary peak table is automatically generated during a search using the "Standard" algorithm. The second algorithm "Use existing Peak Table" employs existing peak tables, allowing one to suppress, for example, solvent peaks.
- *Search Sensitivity:* Using *Search Sensitivity* you can control the result of your search. It is difficult to give a general recommendation for the sensitivity value, because the search result is highly dependent on the type of recorded spectra. For spectra taken from KBr pellets with a typical signal-to-noise ratio, a sensitivity setting of 6 to 10 would be reasonable. The best is, however, to learn about different sensitivity settings by doing test runs applied to known substances. As a rule of thumb, values higher than 15 rarely yield a significant result.



a



b

Figure 11.26. The *Spectrum Search* dialog box: *Use Search Report for Searching* (a) not checked and (b) checked.

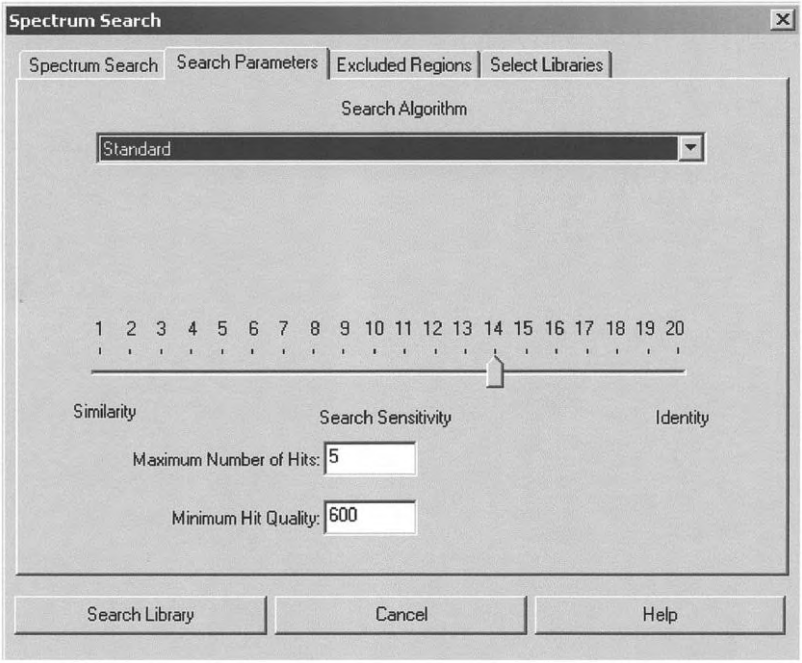


Figure 11.27. The *Spectrum Search* dialog box: *Search Parameters* page.

- *Maximum Number of Hits*: The number of hits that are to be saved in the search report can be specified. Depending on the value set in the *Minimum Hit Quality* field, the resulting number of hits can be lower than this value.
- *Minimum Hit Quality*: This field is used to enter the minimum value to qualify as a hit in the search. A value of 1000 would be a perfect match, whereas a value of zero is obtained in the case of no correlation at all. In reality the search algorithm gives a value higher than zero even if no or negligible similarities exist between spectra. Therefore, one restricts the number of hits included in the search report to a lower limit; the default setting is 300. This value also depends on the sample type and should be evaluated by measuring representative samples. Only if a search yields no hits, should the *Minimum Hit Quality* be set to a higher value.

On the *Excluded Regions* page (Fig. 11.28) you can limit the search to certain wavenumber regions of the spectrum instead of comparing the whole recorded range of the spectrum to the library spectra. Enter either the spectral ranges manually in the table cells or use the *Interactive Range Selection* button to directly select a range in the spectrum. Once you have defined a list of regions to exclude you can save them for future use by clicking on *Save Ranges as*. Using the *Clear Ranges* button clears the table and the *Restore Last Ranges* button automatically calls up the last range definition you used.

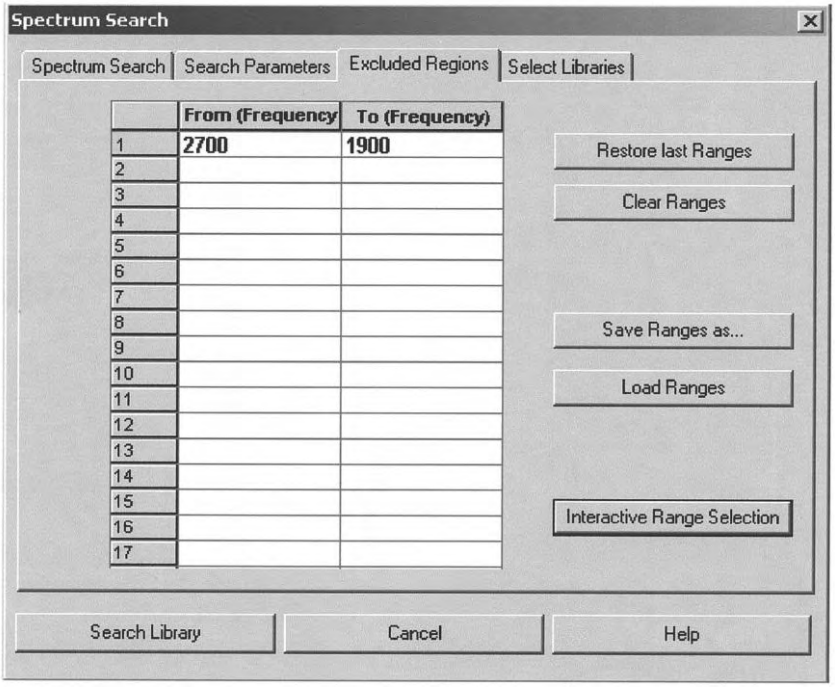


Figure 11.28. The *Spectrum Search* dialog box: *Exclude Regions* page.

On the *Select Libraries* page (Fig. 11.29) you select at least one library to apply for the search. A search can involve either one or more libraries, but creates only one search report.

Use the *Add Libraries* and *Remove Libraries* buttons to include or exclude libraries in the list. You can also save your library file selection for future use and recall it using the *Change List* button. After loading a library list, the library files included in the list are marked by a small bullet.

You can also test the integrity of a library file with the *Check Libraries* command. This command also updates the number of file entries. If a file passes the test, it will be checked by a green mark.

The result of a spectrum search will be saved in the report data block SEARCH. By right-clicking on this block you have access to a short report of spectrum research; an example is given in Fig. 11.30. By double-clicking on the search block you can open an extended report in a *Library Window* Library Wind... . If you check the *Show Search Report Immediately* box on the *Spectrum Search* page, the extended report will be displayed automatically.

The report window shown in Fig. 11.31 consists of four areas, which can be adjusted in size by moving the window bars. The list at the bottom of the window contains all spectra matching the search criteria. In the first column, the hits are

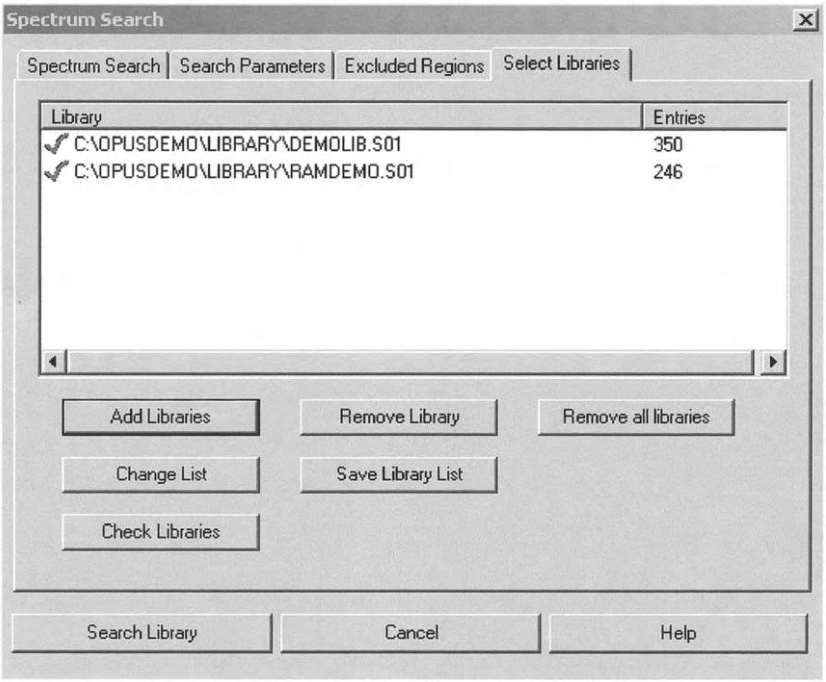


Figure 11.29. The *Spectrum Search* dialog box: *Select Libraries* page. The green mark indicates the integrity test passed.

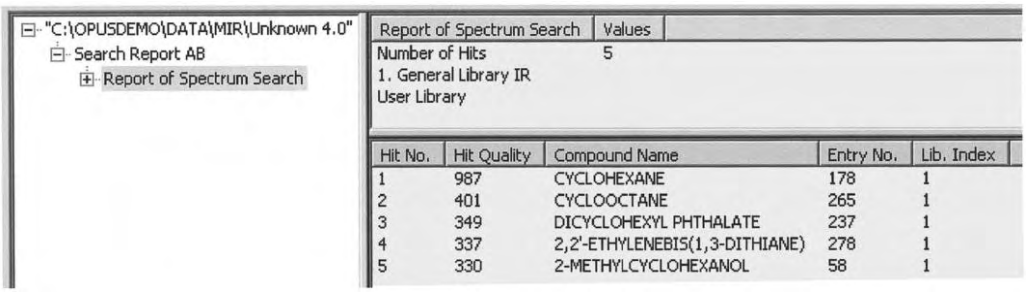


Figure 11.30. The short report of the spectrum search for the sample "Unknown 4" based on the standard algorithm with the following parameters: search sensitivity 14, maximum numbers of hits 5, minimum hit quality 300, and no excluded regions.

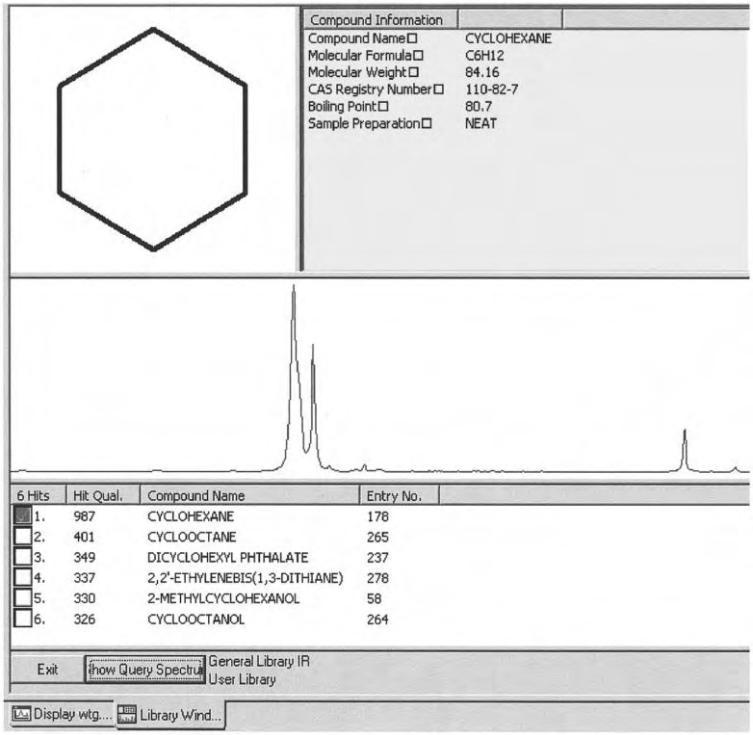


Figure 11.31. The extended report of a spectrum search.

numbered and sorted by hit quality. The first spectrum is selected (red square) and automatically displayed in the display area above. The spectrum will only be displayed, if the box before the spectrum name is checked. To display the substance information and the structure in the two upper areas, the spectrum must be selected. The hit quality is marked before the name of the substance, followed by the entry number of the spectrum in the library.

To illustrate the approach to identifying an unknown material, the search results concerning the file MIR\Unknown 4 based on the IR library are shown in Fig. 11.30 and 11.31. Obviously, this spectrum belongs to the compound cyclohexane.

When several hit numbers are checked in the search report, it is also possible to display together several spectra (see Fig. 11.32). Of course, the substance information and the structure are only given for the hit number marked in red.

Display the query spectrum by clicking on the *Show Query Spectrum* button. Close the report window by clicking on *Exit.*

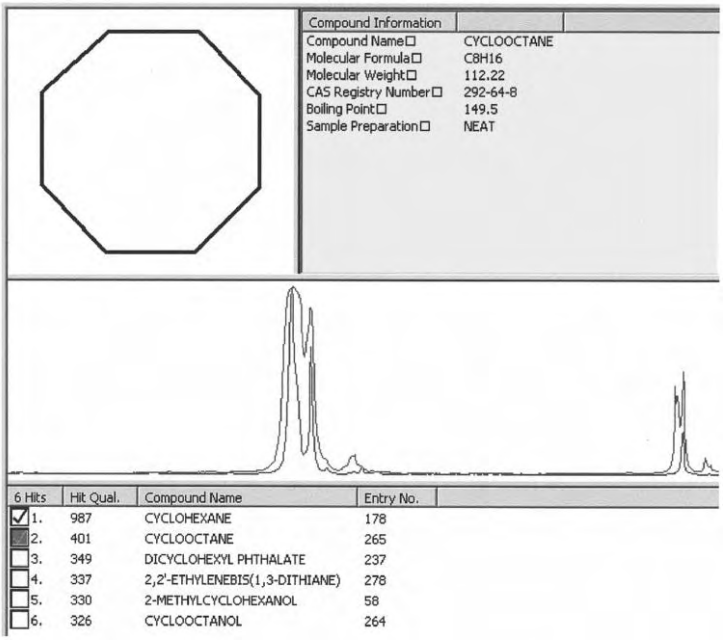


Figure 11.32. The extended report of a spectrum search with two spectra shown.

11.6 Editing Libraries

The Library Editor comprises all the tools needed to maintain your own spectral library, named *User Library*. Open the three pages dialog box with the *Library Editor* command from the *Evaluate* menu or click on the equivalent icon.

The box in the upper part of the *Edit Library* page displays information about the currently active library (see Fig. 11.33). Here you find the library's name and path, the description of the library and information about the number of entries, both valid and deleted entries. You can change the active library by clicking on the *Change Library* button; that means, based on the demo version, you can select either the IR library "Demolib" or the Raman library "Ramdemo".

The first page also lists the library processing functions: *Load Entry, Delete Entry, Change Description,* and *Change Info Definition.*

The function *Load Entry* extracts an entry from the library and saves it as an OPUS file labeled ENTRY*n*.0. Here, *n* represents the entry number in the library; this value must be stated. You can enter this number either by hand or by double-clicking on the entry number in the *Library Entries* page. When you click on the *Edit* button, the respective spectrum is displayed in the spectrum window and the spectral data file ENTRY*n*.0 appears in the browser

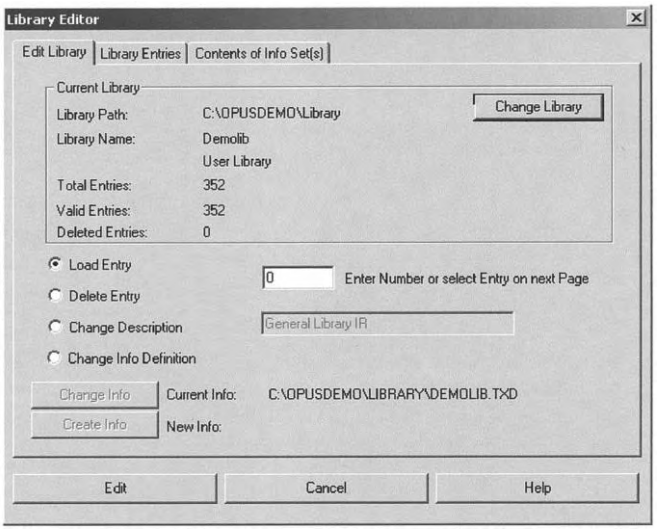


Figure 11.33. The *Library Editor* dialog box: *Edit Library* page.

window. The file ENTRY*n*.0 contains all data blocks saved in the entry, these are for the IR library the IR absorption spectrum, the molecular structure, and the information block, and for the Raman library the Raman spectrum (unfortunately marked as *AB*) and the information block.

Use the function *Delete Entry* to delete an entry from the library. Again, you first have to state an entry number. The number of deleted entries will be displayed in the library information section on the upper part of the page.

If you want to change the description of the library, select *Change Description* and enter the new description in the field on the right side.

The function *Change Info Definition* allows you to change the info mask associated with a library. Either choose an existing mask by clicking the *Change Info* button or create a new mask *(Create Info* button). However, the last function should only be used to append lines to an existing mask or to edit typos in an existing mask. Use the *Setup Info Mask* function from the *Edit* pop-up menu to create a new info mask (see Section 7.3)

The *Library Entries* page as shown in Fig. 11.34 lists all entries of the active library. Together with each entry, the entry number, the compound name, the molecular formula, and the molecular weight as well as the CAS Registry Number are displayed. Double-clicking on an entry number automatically copies it to the input field of the first page whereupon the first page will automatically be displayed.

In case you deleted one or more entries, this will be marked next to the number of the deleted entry.

The *Contents of Info Set(s)* page displays the info text definition of the selected library. Figure 11.35 depicts the info set used for the IR library. If you selected *Change Info Definition on the Edit Library* page, a new info mask

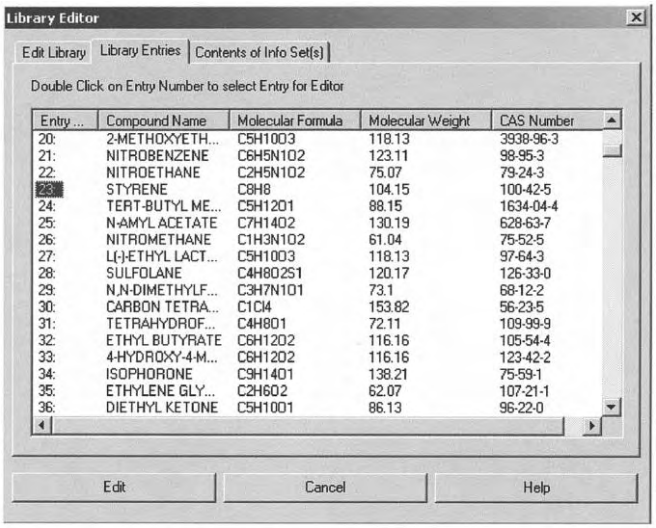


Figure 11.34. The *Library Editor* dialog box: *Library Entries* page.

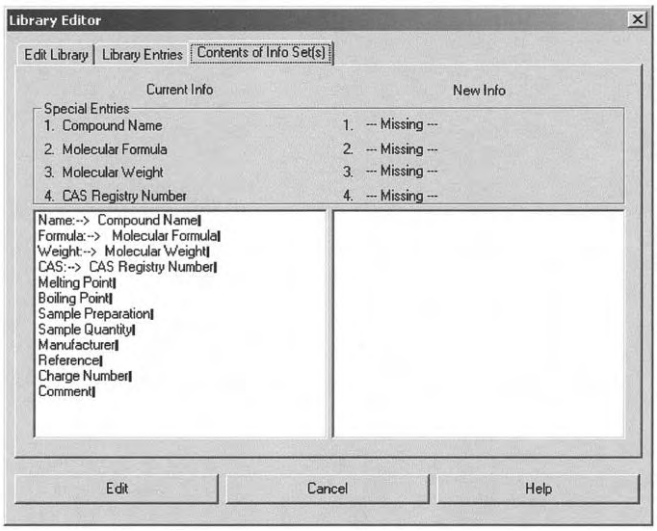


Figure 11.35. The *Library Editor* dialog box: *Contents of Info Set(s)* page.

will be displayed in the right column (see Fig. 11.36). Check to see that both the new and the old masks are compatible; the new mask must contain all fields included in the old mask but may also list additional fields.

Fields that will be assigned automatically to the special entries of a library, namely *Compound Name, Molecular Formula, Molecular Weight* and *CAS Registry Number* are stated above the lists; the corresponding entries are also marked in the lists.

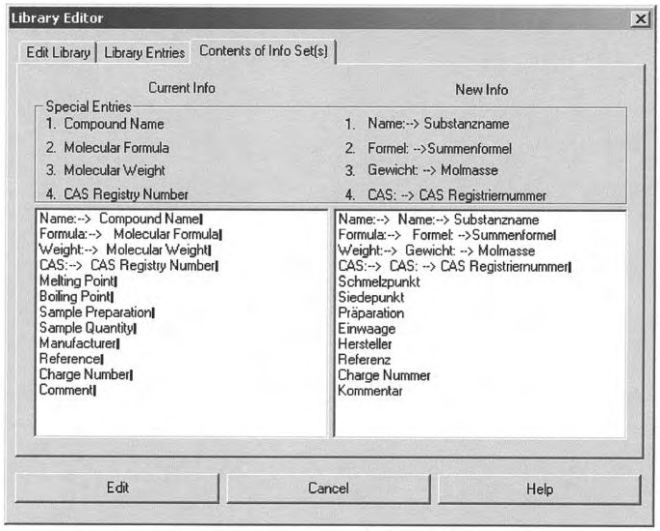


Figure 11.36. The *Library Editor* dialog box: *Contents of Info Set(s)* page with a new info mask.

11.7 Library Browser

This function is a very useful tool for having an overall view of which spectra are available in a library.

By clicking on the corresponding icon you have access to the appropriate library window that comprises four areas. Then click on the ⊞ icon in the top left corner of the left pane to list the compound names of the library entries. To display the spectrum, the compound information, and the structure, select the compound desired. As an example, all these data for methyl formate are shown in Fig. 11.37.

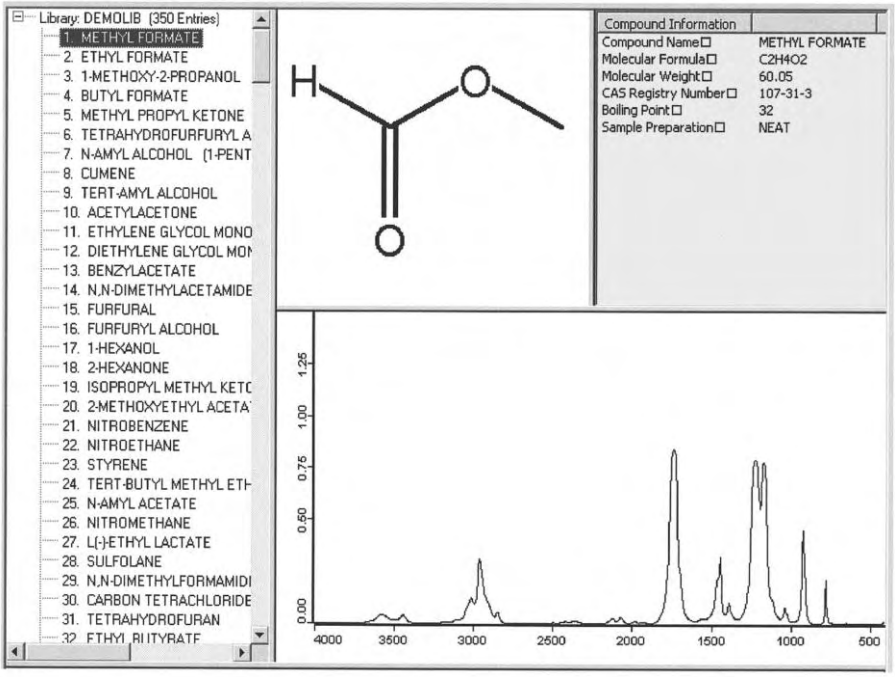


Figure 11.37. The *Library Browser* listing the entries of the IR library. Data for the first entry METHYL FORMATE are shown.

12 Display

The commands within this pop-up menu (Fig. 12.1) manipulate the display of the active spectrum window. They allow you to zoom in on a detail of the display, and go back to the original display size. Some of the commands are not always available, depending on the preceding steps performed. For example, the *Forward* and *Back* commands are only active if you switched the display magnification.

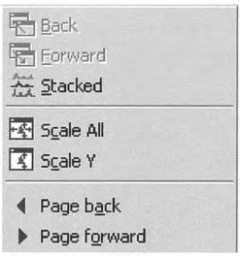


Figure 12.1. The *Display* pull-down menu.

12.1 Back and Forward

Back allows you to restore the original display size on your screen after you have zoomed in on a detail of the displayed curve.

Forward will display the zoomed in area again.

12.2 Stacked

If you load more than one spectrum into the same spectrum window, you have the option of a stacked display i.e. the spectra will not overlap. However, the overview window is not affected by this command.

Figure 12.2 displays the IR spectra of benzene and nitrobenzene in (a) normal view, (b) stacked view, and (c) stacked view with an additional abscissa. As you can see, the ordinate will be duplicated when displaying two spectra in the

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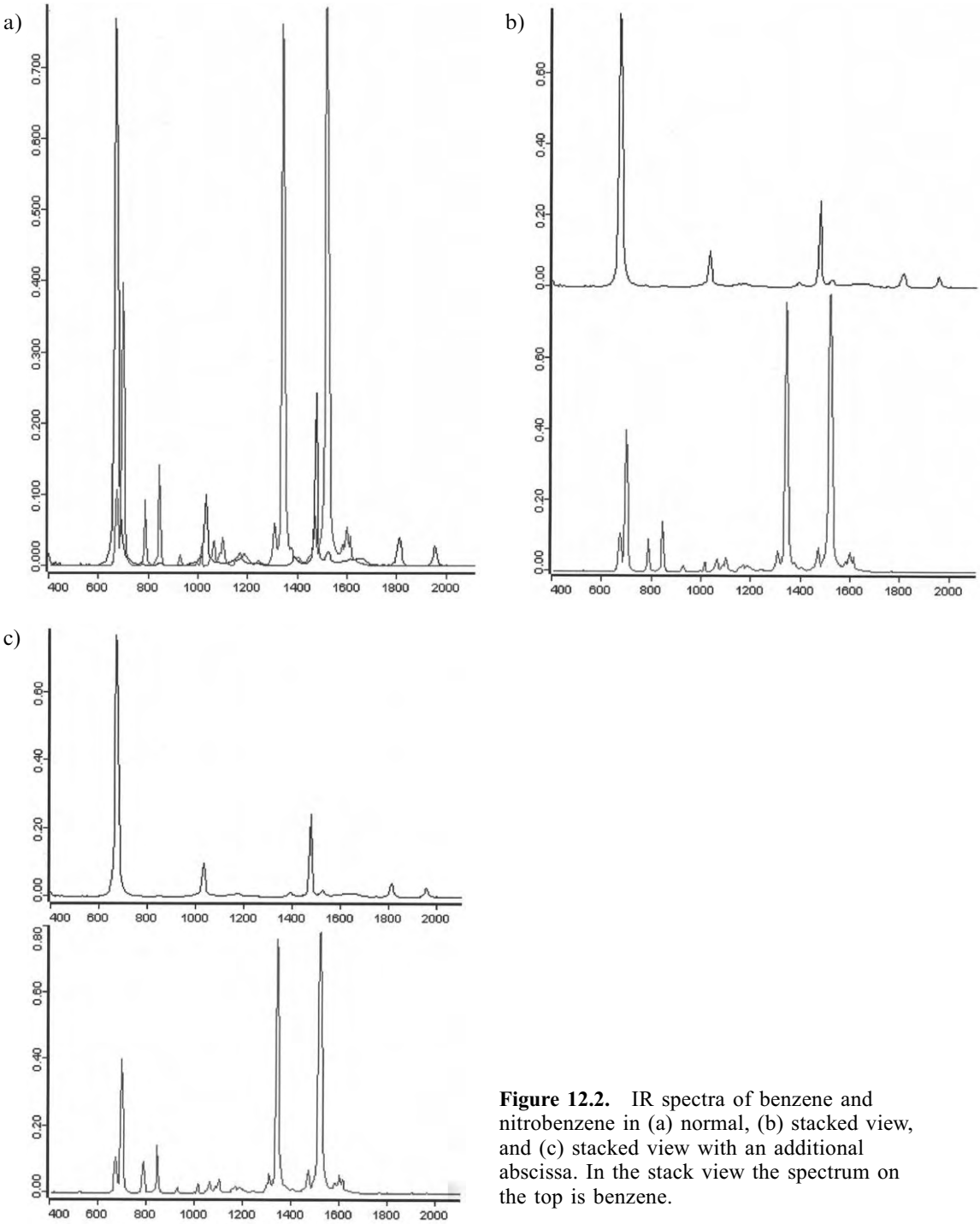

Figure 12.2. IR spectra of benzene and nitrobenzene in (a) normal, (b) stacked view, and (c) stacked view with an additional abscissa. In the stack view the spectrum on the top is benzene.

stacked mode. In addition, you can separate both spectra by displaying another abscissa, using the *Axis* command of the spectrum window pop-up menu, which is accessible by right-clicking on the spectrum window (see also Section 3.3). If you expand a part of one stacked spectrum, the other spectrum will automatically be enlarged.

12.3 Scale All and Scale *Y*

Using the *Scale All* command, you can scale the entire spectrum to the spectrum window.

If you only want to scale the ordinate, you can achieve that with the *Scale Y* command. If you are in the *Stacked* mode, every spectrum will be scaled to fit its region of the display.

12.4 Page Forward and Page Backward

and If you have displayed only a part of a spectrum, you will see this part shown on a white background in the overview while the rest of the spectrum that is not shown appears on a gray background. You can move the displayed region along the wavenumber axis using the *Page Forward* and *Page Backward* commands. In this case you can browse a spectrum while keeping the displayed frequency region the same.

13 Print

The *Print* pull-down menu (Fig. 13.1) contains the functions for printing spectra and reports. However, before you can start to print spectra you first have to define a printer. We assume that a printer is already installed on your computer.

Figure 13.1. The *Print* pull-down menu.

13.1 Defining Print Parameters

Use the *Print Setup* command from the *Print* menu to define the printer and the print parameters like paper size and print quality (see Fig. 6.7). Clicking on the *Properties* button opens another dialog box. The settings shown will depend on the model of your printer.

13.2 Print Spectra

The command *Print Spectra* uses a template to create a plot of spectra and all associated parameters. After loading as usual the relevant spectrum into the window of the *Select Files* page of the *Plot Spectra* dialog box (Fig. 13.2), it is necessary to choose a page layout. Hence, use the *Change Layout* button to select a template from the folder OPUSDEMO\Scripts (see Fig. 13.3). To inspect the final layout prior to plotting, click on the *Preview* button to open another window that shows an exact copy of the subsequent plot. An example of such a print preview is displayed in Fig. 13.4.

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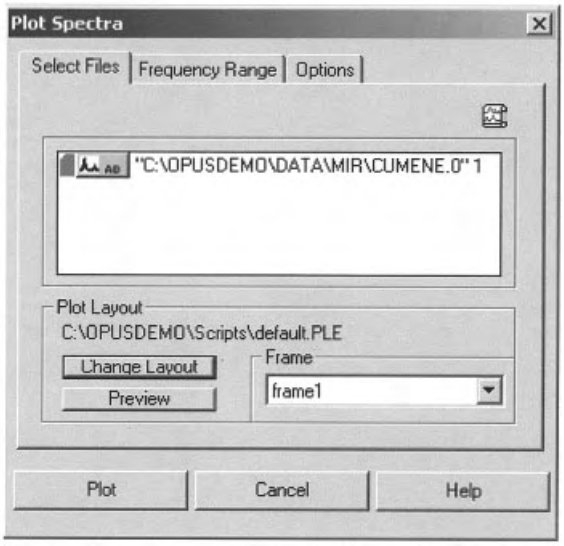

Figure 13.2. The *Plot Spectra* dialog box: *Select Files* page.

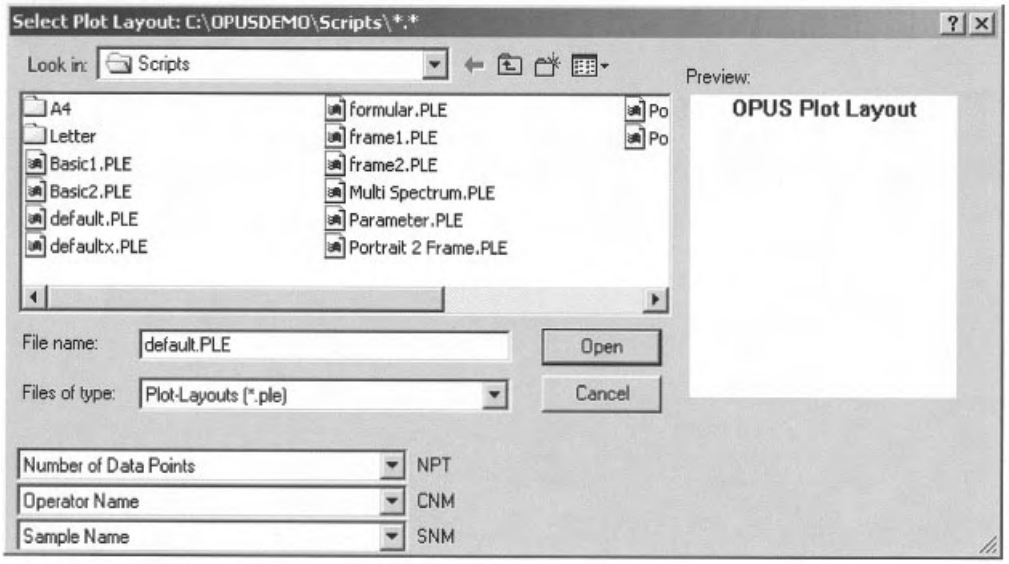

Figure 13.3. The *Select Plot Layout* box.

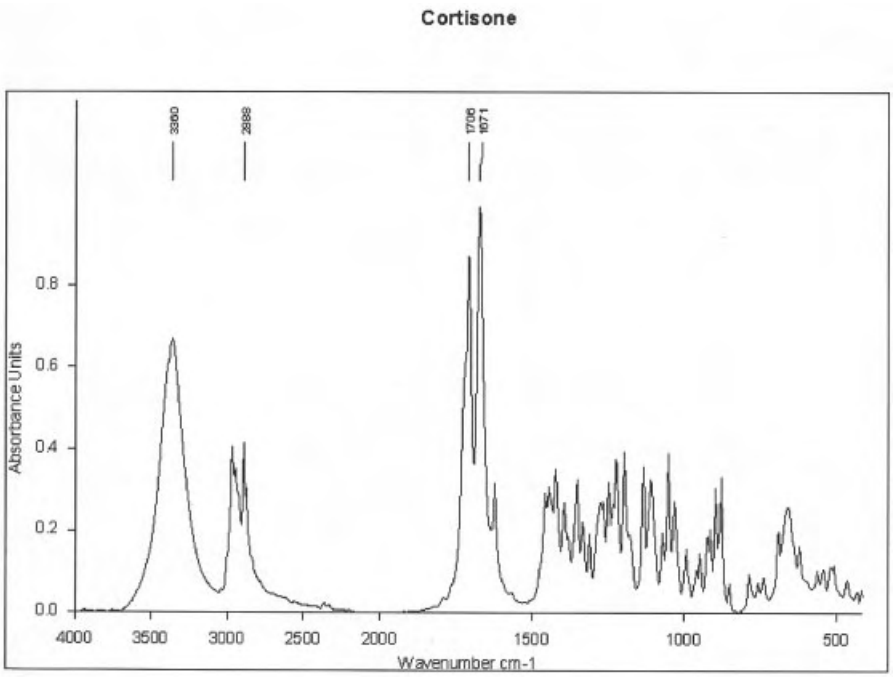

Figure 13.4. Print preview of the IR spectrum of cortisone using the template *Basic1.PLE*. On the *Options* page the items *Auto Scale to All Spectra* and *Use Compressed Wavenumbers* were checked.

In addition, you can specify the spectral range of interest on the second page of the dialog box (Fig. 13.5). Furthermore, on the *Options* page shown in Fig. 13.6 the number of peaks to be labeled and the scaling of the *x*-axis are defined. If you check *Auto Scale of All Spectra*, the file limits are used. Otherwise, the limits as specified on the *Frequency Range* page are used. You can choose between a linear *x*-axis and an axis with compressed wavenumbers. If *Use Compressed Wavenumbers* is marked, the wavenumbers above 2000 cm^{-1} will be compressed by a factor of two.

OPUS offers various plot layouts saved in the directory OPUSDEMO\Scripts. Find out for yourself which template best meets your needs.

Using the plot layout *Scripts\A4\structure.PLE* it is also possible to plot the chemical structure of the sample, which of course must be available. An example is depicted in Fig. 13.7.

Instead of a printout, the print job can be sent to the clipboard for further processing using another program.

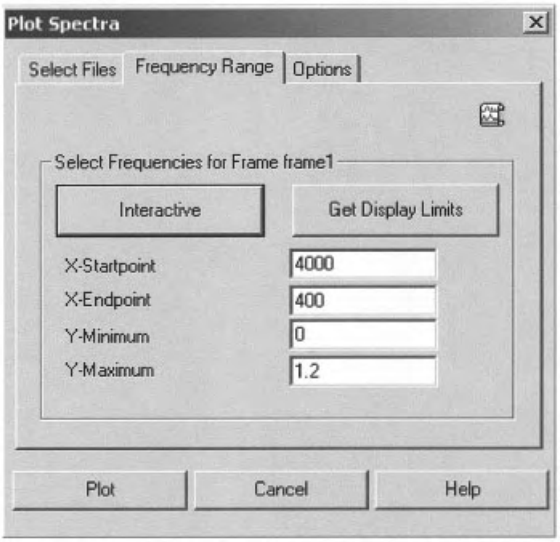

Figure 13.5. The *Plot Spectra* dialog box: *Wavenumber Range* page.

Figure 13.6. The *Plot Spectra* dialog box: *Options* page.

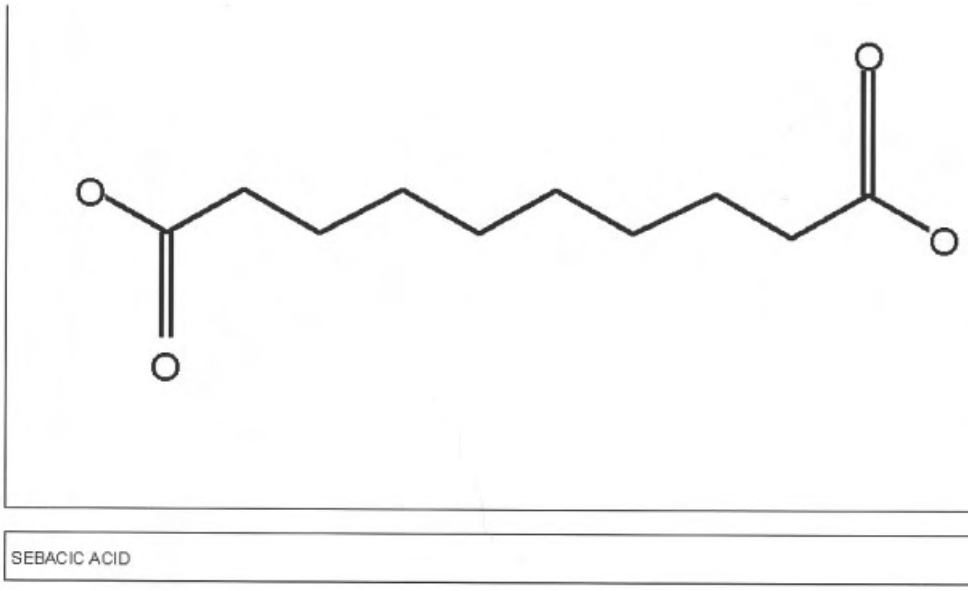

Figure 13.7. The chemical structure of sebacic acid plotted using the template *Structure.PLE.*

13.3 Quick Print

You can use *Quick Print* to plot the contents of an OPUS view without specifying a template. In this case the plot layout *default.PLE* will be used.

The *Quick Print* command generates a printout showing the spectrum window as it is, i.e. if you only display part of a spectrum then only this part will be printed, including all labels and annotations.

13.4 Print and Print Preview

The commands *Print* and *Print Preview* are only available if a report window is open. You can view the result of a printout prior to printing by using the *Print Preview* command, as the name indicates. Clicking on *Print* will start the printing process immediately.

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